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JOURNAL OF ANATOMY

MATURATION OF REGENERATING NERVE FIBRES WITH VARIOUS PERIPHERAL CONNEXIONS

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INTRODUCTION

During recent years it has become apparent that the size reached by regenerating nerve fibres, though dependent partly on the diameter of the parent fibres in the central stump (Hammond & Hinsey, 1945; Simpson & Young, 1945), is mainly influenced by factors peripheral to the point of injury (Sanders & Young, 1944). The diameter of the tubes into which the new fibres grow may, in some circumstances, produce a restriction on the growth, but it is now clearly established that the most powerful peripheral influence is the contact which is made with end organs (Weiss & Taylor, 1944; Sanders & Young, 1945; Weiss, Edds & Cavanaugh, 1945). Regenerating nerves which have been disconnected from their end organs develop many small fibres. Connexion with the periphery leads to an increase in size and reduction in number of both sensory and motor fibres (Sanders & Young, 1946).

There is at present no information as to how this influence is exerted by the periphery on the growing nerve fibre. We do not even know whether mere contact with the end organs is able to produce the effect, or whether active function is necessary. It is clear that for further progress exact quantitative treatment is necessary; we must know the extent to which the nerve fibres have grown following various procedures. In recent work (Sanders & Young, 1945, 1946) measurements and counts have been made of the fibres in the peroneal nerve of rabbits, but as this contains more than 5000 fibres and has both motor and cutaneous branches it cannot be used conveniently for extensive experiments. A much more suitable nerve is that which supplies the medial head of the gastrocnemius, since this only contains about 400 medullated fibres and runs unbranched for several centimetres. The fact that this, like other muscle nerves, contains sensory as well as motor fibres is an unpleasant complication of the experiments which could only be surmounted by deafferentation. This we have not felt it wise to undertake in animals which must be dealt with alike on both sides and must survive for long periods.

We have used this nerve to determine the effect of various types of peripheral connexion on the process of maturation. The fibres were interrupted by crushing the nerve as high as possible, giving a length of several centimetres in which the fibres were regenerating. By severance low down and union with various nerves, answers were sought for the following questions:

(1) Is there any difference in the size reached by fibres connected with their own and with strange muscles?

(2) Does union with muscles reduced by the atrophy of denervation or of tenotomy have the same effect as union with unwasted muscle?

(3) What is the effect on maturation of union of motor fibres with sensory end organs?

(4) If no union with the periphery is allowed, do the fibres gradually increase or decrease in number or diameter?

(5) If fibres have been allowed to regenerate for some time without any peripheral connexion, have they still the full power of increase in diameter if later allowed to make connexions?

Experiments on nerve maturation must all be of long duration, and if a sufficient number are to be undertaken to provide information about this remarkable influence of the periphery, it is important first to establish whether the phenomenon can be detected in the earlier stages. Gutmann & Sanders (1943) have shown that regeneration only approaches completion after 250 days in the crushed peroneal nerve of the rabbit, and after severance and suture is still incomplete after one year. If all experiments on nerve maturation must last a full year or more it will be very difficult to undertake them in variety sufficient to answer any questions. We have therefore limited our animals to 100 days of regeneration. The results have enabled us to give clear answers to some of our questions, and suggest that it may be possible to plan future experiments to last for this short period, though no doubt investigation of the later stages of regeneration must also eventually be undertaken.

METHOD

The nervus gastrocnemii medialis (n.g.m.) of the rabbit separates from the remainder of the sciatic in the upper or middle portion of the thigh. It lies loosely bound to the main tibial trunk between that nerve and the sural, gradually becoming more fully separated and finally dividing into two small branches as it approaches its muscle. By drawing aside the nerves on either side of the n.g.m. with fine hooks it can be dissected free for a length of up to 4 cm. in favourable cases.

For determination of the sizes and numbers of fibres in the nerve the method described by Sanders & Young (1946) was followed. The nerves were fixed lightly stretched on cards in Flemming's fluid, sectioned at 5μ in paraffin, stained by Weigert's method and photographed at a magnification of $750\times$ direct on to bromide paper. The fibres were then marked off in size groups of 2μ by callipers, each fibre being pricked as counted, by a needle made to actuate a relay and hence to serve as a counting device. In every case all the medullated fibres in the nerve were counted.

Any method which involves measuring living tissues after removal from the body is liable to errors produced by swelling, shrinking or changes in shape as

the very soft material is transferred to strange fluids. The main factors influencing the estimated diameter of the fibres are:

(1) *Stretching and drying during removal.* The magnitude of influence from these sources is unknown.

(2) *Fixation undoubtedly changes the diameter of nerve fibres.* We experimented with various osmium-containing fixatives and found the most consistently satisfactory to be: 1 % H_2CrO_4 in distilled water, 15 c.c.; 2 % OsO_4 in distilled water, 4 c.c.; CH_3COOH (glacial), 2 drops. The mixture *must be freshly prepared* and deviations from its composition will give marked variation in result. The nerve must be cleaned of fat as far as possible before fixation, otherwise the fibres close to fatty tissue will be poorly fixed. With this fluid most of the fibres can be fixed approximately as spheres, though never without some irregularities (Pl. 1, fig. 1); some fibres always become oval or crenated. Even with a nerve as small as the n.g.m. the fixative does not always fix the nerve uniformly and if any tissue is left adherent to the nerve there will be distortion of the fibres at the centre of the piece. In order to allow uniform penetration of the fixative the nerve should be stretched across a hole cut in the card to which it is attached.

With these precautions and careful handling of the tissue after fixation, specimens can be obtained in which it is reasonable to suppose that the diameters of the fibres are 10 % less than those found in life (see Gutmann & Sanders, 1943 for discussion). It must be emphasized, however, that this figure is largely a guess. No one knows the diameter of living nerve fibres or whether the subsequent treatments affect large and small fibres differentially.

Other techniques tried gave less satisfactory results. Formol does not give uniform or good preservation of fibres. 1 or 2 % osmium tetroxide, in distilled water or 0·9 % NaCl, gave satisfactory fixation only of the outer fibres, and osmium vapour was no better. Variations of Flemming's fluid were tried in which the solutions were made up with 0·9 % NaCl instead of distilled water, with and without the addition of acetic acid. The mixtures without the acetic acid were found less satisfactory than those with it and the presence of the sodium chloride with acetic acid produced nerves with more distortion of fibres at the centre. The worst result was given by mixtures containing neither salt nor acid. For the probable reasons for these variations see Young (1935). It must be emphasized that the variations are considerable and that careful attention to the preparation of each batch of fixative is essential for exact work.

(3) *Errors of measurement.* Care was taken to avoid errors in the magnification at which the photographs were taken and they do not exceed 0·5 %. The actual measurement of the fibres involves many approximations; only rarely are they perfectly round, and when markedly oval the mean of the greatest and least diameters was taken. Crenated fibres were supposed to be shrunken by 10 % and therefore referred to a size-group one higher than that given by their diameter (see Duncan, 1934).

Accurate measurement and counting are especially difficult for the small fibres and hence in those regenerated nerves which contain many of them (see Pl. 2, figs. 6, 7) considerable errors are possible in the 0–2μ group and care must be taken only to consider as fibres those with a white rim and dark centre so as to avoid inclusion of other objects which may have been stained.*

Counts were made of three of the normal nerves by two observers and showed divergences of 5, 9 and 25 in the number and 0·09, 0·18 and 0·54 in the root mean square diameters of the fibres (p. 5). These differences in estimates of diameter no doubt arise from different systems of approximation. The readings of the two observers are scattered at random through the results and no account has been taken of this factor.

(4) *Breed, age and weight of animals.* These, no doubt, affect the fibre diameters, but no account has been taken of them here. All animals were above 6 months old and were kept under similar conditions with no exercise outside their cages. Further experiments are now being undertaken to determine whether exercise affects the maturation of the nerves.

It is clear from these considerations that it cannot be claimed that we have made an exact estimate of the diameters of the fibres in living nerves. The sizes given by our estimates may be less than those in the living state by a factor of about 10%. Uncertainty about this figure does not, however, affect the conclusions, since all nerves were treated alike and there is no reason to think that variations due to the above sources are larger than 5% or are not distributed at random through the results. With the exact methods now available it will be important to standardize all these factors in investigations of this type. The consistency of the results is not unsatisfactory, but could be improved.

A most serious variable to be controlled in all experiments on nerve regeneration is the nature of the lesions interrupting the nerves and especially the success with which severed stumps are united. The upper lesion in this series was in every case a single crush made with smooth-tipped watch-makers forceps, held tightly together for 10 sec. by the clock. Variations even in this simple lesion no doubt occur, but it can be regarded as constant for our purposes. Sections of the injured point at biopsy 100 days later show a slight swelling and a few fibres running obliquely, but no gross interference with the longitudinal pattern of the nerve (Pl. 1, fig. 2).

Severance of the lower region of the nerve was by a single scissors cut, and every care was taken not to damage the severed ends, which were never seized with forceps. Union of stumps was made with concentrated cockerel plasma, for the supply of which we are indebted to Mr P. B. Medawar. With all precautions it remains very difficult to make standard unions of small nerves, and the experiments leave much to be desired in this respect. In order to express the closeness of union we fixed the specimen at autopsy in Flemming's fluid, sectioned it longitudinally in paraffin, stained with Weigert and photographed the union at a magnification of 20×. The extent of the separation

* The figures in the plates are positives whereas the counts were made on negative prints.

of the two stumps was then measured, using the point of maximal swelling of each stump (Pl. 1, fig. 3), as a reference point. This figure of course exaggerates the actual distance between the cut surfaces.

Composition of the nervus gastrocnemii medialis

Table 1 shows the distribution of fibres in nine nerves from rabbits of the various breeds and weights.* Two counts each were made of the nerves of animals 1619 and 1620, one piece being taken as high as possible and the other immediately above the point of division of the nerve. The number of fibres does not increase over this stretch of nerve, nor do the fibres decrease in diameter passing downwards.

As in other muscle nerves, the distribution of fibre size is markedly bimodal with peaks at about 7 and 16 μ and few fibres around 10 μ. Eccles & Sherrington (1930) showed that the n.g.m. of the cat, which contains between 600 and 1000 fibres, has a similar bimodal distribution and that both the large and small groups contain sensory and motor fibres. For the purpose of our study it is important to make comparisons of the diameters reached by the regenerating fibres after various procedures. The average fibre diameter is not a suitable value for this purpose and we have therefore adopted the procedure already used (Sanders & Young, 1946) of calculating the root mean square of the population of diameters. This figure, D, gives us an estimate of the diameter of a 'typical' fibre of the nerve, such that a number equal to that present in the nerve and all of this diameter would have the same volume of axoplasm + myelin per unit length as is actually found distributed over a range of fibre sizes.

The mean of the nine counts (from 7 nerves) shows 399 fibres (N) and a root mean square diameter (D) of 13·26 μ. Variations cannot be clearly correlated with any single factor but the young animal (1620) has few fibres, especially in the small size groups, to which fibres are probably added later in life. The two large animals (1628 and 1629) include almost the lowest as well as the highest figures for N. It should perhaps be stated that these two animals were subjected to tenotomy of the opposite tendo Achillis (see p. 11) and the nerves are therefore not strictly those of normal animals. It may be noted that in all cases the upper end of the distribution cuts off rather sharply at 20 μ.

RESULTS

A. *Maturation of nerve after union with various muscles*

(1) *Union with its own muscle*

Table 2 (*a*) shows the number and root mean square diameter of the fibres regenerated 100 days after crushing the n.g.m. as high as possible and at the same operation, cutting it and suturing it below into its own peripheral stump.

* The numbers indicate individual animals, the letters following them the separate pieces of tissue removed for fixation. Thus 1619c is piece 'c' of animal 1619 and 1619d is a separate piece which may be another part of the same nerve or a part of the nerve on the opposite side.

Table 1. *Distribution of fibres in normal nerves of the rabbit*

N is the total number of fibres in the nerve; *D* the root mean square diameter (μ).

Nerve	Breed of rabbit	Wt./lb.	Level	Diameter μ 0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	*N*	*D*
				(*a*) Nervus gastrocnemii medialis												
1609c	Blue Beveran	5	Low	1	14	35	49	35	20	37	62	136	27	0	416	13·68
1610a	Butterfly	4¾	Low	5	13	31	48	45	16	57	154	58	9	0	436	12·94
1611a	Butterfly	4	Low	3	10	30	53	27	35	56	121	62	5	0	402	12·84
1619c	Wild Type	3½	Low	9	18	38	41	19	30	53	118	56	10	0	392	12·74
1619d	Wild Type	3½	High	1	15	37	45	23	22	43	104	84	11	0	385	13·16
1620c	Young Flemish	4	Low	2	11	24	53	23	13	27	46	111	43	1	354	14·03
1620d	Young Flemish	4	High	2	8	35	53	27	15	35	57	125	26	0	383	13·69
1628a	Grey	8½	Middle	6	19	23	33	17	11	20	104	104	19	0	356	13·90
1629a	Wild Type	8	Middle	9	20	40	49	30	26	100	159	32	2	0	467	12·40
														Mean	399	13·26
				(*b*) Nervus plantaris												
1663a	Grey	8	—	0	15	31	48	45	56	58	36	253	111	16	669	14·80

Table 2. *Fibres regenerated.* (*a*) *Below crush and above direct suture of nerve;* (*b*) *below crush alone;* (*c*) *below crush and above suture of nervus gastrocnemii medialis to nervus plantaris*

Nerve	Diameter μ 0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	*N*	*D*	Separation mm.
					(*a*) Primary suture of nervus gastrocnemii medialis with itself								
1622h	409	301	137	148	143	16	0	0	0	0	1154	4·87	1·1
1647b	262	567	238	150	128	89	11	1	0	0	1446	5·38	1·7
1653h	56	483	187	122	128	18	1	1	0	0	996	5·30	2·0
1654j	124	399	316	175	122	47	6	0	0	0	1189	5·56	—
1661c	30	369	318	126	107	92	30	2	1	0	1075	6·33	1·6
1693c	105	550	218	103	94	86	11	0	0	0	1167	5·51	—
										Mean	1171	5·49	
					(*b*) Crush of nervus gastrocnemii medialis without severance								
1662f	15	62	101	92	49	101	128	16	3	0	567	9·41	—
1661f	24	157	77	92	58	122	53	10	0	0	593	9·00	—
					(*c*) Union of nervus gastrocnemii medialis with nervus plantaris								
1560b	22	169	218	153	77	104	113	33	0	0	889	8·20	0·8
1574e	19	86	100	171	160	59	7	0	0	0	602	7·40	—
1627j	29	113	65	145	166	69	8	0	0	0	595	7·44	3·5
1663g	15	308	156	117	89	70	5	0	0	0	760	6·15	1·0.
										Mean	712	7·29	

The distances of separation of the stumps shown in the Table indicate that ideal conditions for union cannot be obtained at this operation, because of the retraction of the stumps. Maturation has proceeded further than in the nerves left without any peripheral connexion (Table 4) giving an average D for the six experiments of 5·49 for 1171 fibres as against 4·01 and 1990 for the five unconnected nerves. The probabilities of chance differences of this magnitude (using Fisher's t, 1928) are 0·01–0·001 and $<0·001$ respectively, and we may conclude that the union of a muscle nerve with its own periphery is effective in increasing the diameter and reducing the number of its fibres.

The maturation was, of course, very far from complete (Pl. 1, fig. 4). Only very few fibres over 14μ were found and as Table 2 (*a*) shows, there was no sign of the typical bimodal distribution of fibre sizes. By comparison with the unsutured nerves of Table 4 (*a*) it is evident that the maturation had produced more fibres in the groups above 8μ and fewer in the 0–4μ range. It is interesting to compare the degree of maturation with that following a single crush high up in the nerve, without any lower interruption. As Table 2 (*b*) shows, the average diameter reached by the fibres after 100 days is then very much greater and the number of fibres has already become reduced almost to normal. Distinct signs of bimodality are present. There are more large fibres than in Table 2 (*a*), while equally striking is the fact that there has been a very drastic reduction of the small fibres. The full importance of the difference between the results shown in Table 2 (*a*), (*b*), is that the same lesion has intervened between the nerves and their cell bodies in both cases. The only differences in treatment of the nerves lie *peripheral* to the level recorded. This shows very strikingly how fibre maturation is controlled by peripheral rather than central influences.

(2) *Union of nervus gastrocnemii medialis with nervus plantaris*

The nerve to the large plantaris muscle of the rabbit separates from the remaining tibial branches low in the thigh and runs close to the n.g.m. Cross union of the two is therefore easy, in fact, easier than the union between the n.g.m. and its own peripheral stump. The operation was satisfactorily performed in four cases and Table 2 (*c*) shows that the diameter of the fibres produced was greater and their number less than after suture of n.g.m. to itself.

The probability that these divergences are due to chance lies between 0·01 and 0·001 for both N and D and they may therefore be regarded as significant. The figures in the last column show that some of the unions were closer in the plantaris sutures but this does not seem to be the explanation of the better maturation. For nerve 1627j showed a very poor union with plantaris, but a good maturation. The explanation for the difference cannot yet be given with certainty but may lie in the greater number of fibres and greater size of fibres in n. plantaris, as shown in Table 1 (*b*). Moreover, in three of these cases (not 1663g) there had been some escape of fibres from the union to regain the peripheral stump of n.g.m. Stimulation of the central stump of

n.g.m. gave contraction of m. gastrocn. med. as well as of m. plantaris. It is certain, therefore, that the regenerating nerve carried a greater peripheral load in these experiments than after suture of n.g.m. to itself (Table 2 (*a*)) and it seems likely that this was responsible for the greater diameter and also, paradoxically, for the lesser number, of the fibres regenerated.

It will be noticed that the difference between the distributions of Table 2 (*a*) and (*c*) is mainly that the nerves in the latter contain fewer small fibres and only to a slight extent do they include more large ones in the groups above 10μ. The upper limit of fibre size reached is similar in the two cases (see below).

(3) *Union of nervus gastrocnemii medialis with nervus solei*

The nerve to the red solei muscle of the rabbit also separates low down in the thigh and the n.g.m. can be joined to it. Table 3 (*a*) shows that good maturation occurred after the operation. Unfortunately, however, adequate precautions were not taken to avoid union with the other tibial branches and at biopsy stimulation of n.g.m. centrally gave contraction of both medial and lateral heads of gastrocnemius as well as of soleus. It seems likely, therefore, that the peripheral load carried by the nerve was greater than in direct suture of n.g.m., and detailed consideration of the effect of the fibre structure of n. solei is not profitable.

(4) *Union of nervus gastrocnemii medialis with nervus peroneus*

The motor branches of the peroneal nerve can just be distinguished at a sufficiently high level to allow union with the n.g.m. if the latter is severed very low. As Table 3 (*b*) shows, the maturation was good although the union had been poor. Indeed, the size of the fibres was greater and their number fewer than on the opposite side of the same animal (1653h, Table 2 (*a*)) where the n.g.m. was sutured to its own peripheral stump. The larger value of *D* is, however, again seen to be due more to the lesser number of small fibres than to the greater number of large ones. The upper end of the distribution is not very dissimilar in the two cases. We may suppose that the difference, as in the case of union with n. plantaris, is due to the greater size of the peripheral load provided by union with the n. peroneus. There is no means of knowing the exact size of the portion of the n. peroneus with which the n.g.m. was joined but it may reasonably be supposed to have been larger than n.g.m. itself. The degree of maturation in this case is especially interesting because it is produced by connexion with a muscle antagonistic to that normally served by the nerve. Evidently effective functioning is not a pre-requisite at least for these earlier stages of maturation.

Table 3. *(a), (b), (c), (d), (g), fibres regenerated after crushing nervus gastrocnemii medialis high up and uniting it below with various nerves. (e) Nerves crushed but not cut; (f) no operation on nerve, tendon cut* (see text)

Nerve	Diameter μ 0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	*N*	*D*	Separation mm.
						(*a*) Union of nervus gastrocnemii medialis with nervus soleus								
1692c	46	143	74	76	89	122	78	0	0	0	0	628	8·17	1·5
						(*b*) Union of nervus gastrocnemii medialis with nervus peroneus								
1653c	13	176	126	92	151	41	3	1	0	0	0	603	6·71	2·9
						(*c*) Union of nervus gastrocnemii medialis with pre-degenerated nervus plantaris								
1574j	7	32	53	76	128	151	21	0	0	0	0	468	8·96	—
1627e	103	330	107	105	75	124	33	0	0	0	0	977	6·25	—
						(*d*) Union of nervus gastrocnemii medialis with itself, plus tenotomy								
1647f	338	856	340	171	129	61	7	0	0	0	0	1902	4·81	—
						(*e*) Nervus gastrocnemii medialis crushed but not cut, plus tenotomy								
1662b	13	101	103	100	101	160	28	0	0	0	0	606	8·19	—
						(*f*) Tenotomy alone								
1628c	4	26	25	18	17	8	39	74	109	20	2	342	14·00	—
1629c	11	17	59	50	17	30	66	176	52	8	0	486	12·65	—
						(*g*) Union of nervus gastrocnemii medialis with nervus suralis								
1654e	53	213	208	147	162	15	0	0	0	0	0	798	6·06	0·7

B. *Maturation after union with atrophic muscles*

(1) *Denervation atrophy*

When the n.g.m. is sutured into itself or into the n. plantaris the fibres arrive in the muscle about 29 days after the operation. This figure is arrived at by allowing 5 days to cross the region at which the nerve is crushed, 6 days to travel approximately 30 mm. from the crushed point to that of union, 5 days to cross the latter and 13 days to travel a further 40 mm. and reach well into the muscle. The assumptions used to reach this figure, namely growth at 5 mm./day below the crush and 3 mm./day below the suture, cannot be wrong by more than a few days (see Gutmann, Guttman, Medawar & Young, 1942). After 29 days the muscle fibres will have undergone only a moderate degree of atrophy (see Gutmann & Young, 1944). In order to discover the effect on maturation of contact with more atrophic muscle fibres in two animals, we made an initial operation at which all the branches of the tibial nerve except the n.g.m. were severed and the central stumps excised in such a manner as to prevent all possibility of reunion. The animals were left for 142 days in the case of rabbit 1574 and 100 days in the case of 1627 and a second operation was then performed. The n.g.m. was now crushed as high as possible, also cut low down and joined with the degenerate lower end of the n. plantaris. At the same time nerves of the opposite leg were also exposed and the n.g.m. joined to the undegenerated n. plantaris. After a further 100 days the animals were killed and the nerves fixed in the usual way.

Table 3 (*c*) shows that maturation of the fibres had been effective in both cases, in spite of the fact that the muscle fibres with which they became connected had undergone long periods of atrophy. In the case of 1574j the fibres were actually larger and fewer after the delayed than after the primary suture (Table 2 (*c*), 1574e), in fact this nerve showed more complete maturation than any other crushed and sutured nerve in the whole series, this result being achieved not by the production of especially large fibres but of a large number of 10–12μ with reduction of those less than 4μ to a very small number.

(2) *Atrophy of tenotomy*

If effective functioning is necessary for maturation of regenerating nerve fibres, then tenotomy should influence the process. In rabbit 1647 the n.g.m. was crushed high up, severed and sutured to itself below. At the same time the whole tendo Achillis was cut and 2 cm. removed from it. On the opposite side the nerve was crushed and sutured but the muscle left intact. After 100 days of regeneration a clear difference was still evident between the two sides, the foot with the tenotomy taking little active part in locomotion, being raised later than the other from the ground and executing a flickering motion in mid air. The tenotomy remained effective therefore and the muscle was producing little or no pull on the heel. The gastrocnemius medialis muscle weighed 1·07 g. on the side with the tenotomy, 2·55 g. on the side with the

intact tendon. Stimulation of the n.g.m. caused contraction of the muscle on both sides.

Table 3 (*d*) shows that the maturation of the nerve united with the tenotomized muscle had been very incomplete. The number of fibres was greater and their diameter less than in any other case of direct suture of the n.g.m., including the opposite side of the same animal. The poor maturation is evidenced by both a small number of larger fibres and a very large number of small ones. Tests of the significance of the difference of D and N from the means of fibre diameter and number produced after suture of n.g.m. without tenotomy show probabilities of 0·05–0·02 that the diameter and $<0{\cdot}001$ that the number would occur by chance. Unfortunately, the sections of the site of union were not satisfactory in this experiment but the naked-eye examination at biopsy showed no evidence of marked separation and the nerve immediately below the union gave good sections which were well filled with fibres. This, together with the fact that the medial head of the gastrocnemius contracted on stimulation of the nerve shows that there is no reason to suppose that the union was less satisfactory than in the case of the nerves shown in Table 2 (*a*). This experiment, though an isolated one, therefore suggests very strongly that the continued atrophy imposed by tenotomy diminishes the effect of a muscle on the maturation of a regenerating nerve.

A further test of the effect of tenotomy on maturation was made by interrupting the n.g.m. by a single crush only, on both sides of the animal (1662) and on one side severing the tendo Achillis. After 100 days the medial head of gastrocnemius weighed 3·28 g. on the intact side and 1·14 g. on the tenotomized side. Table 3 (*e*) compared with Table 2 (*b*) shows that the number of fibres was less and their diameter greater on the side without the tenotomy. The largest fibres are only 14μ on the tenotomized side (1662b) as against 18μ on the other; there are considerably more fibres less than 4μ, and there is no clear sign of reappearance of the bimodal distribution which is already marked in 1662f. This experiment is highly suggestive, especially in conjunction with the previous one. The variables possible in the process of crushing the nerve are so small that such marked divergence in the resulting maturation is likely to be due to outside factors.

In view of this influence of tenotomy on regeneration, we considered it possible that section of a tendon would affect the nerve even if the latter were not interrupted. In rabbit 1628 therefore we severed the part of the tendon presumed to belong to the medial head of gastrocnemius without any other operation. 100 days later there were no obvious defects of locomotion and the tendon seemed to have rejoined the other parts of the tendo Achillis. The muscle weighed 3·3 g., that on the normal side 4·5 g. The nerve (Table 3 (*f*)) showed no signs of atrophy compared with that on the opposite side (1628a, Table 1).

In rabbit 1629 the whole tendo Achillis was cut and 100 days later the medial head of gastrocnemius weighed only 1·7 g. as against 4·2 g. on the

opposite side. This atrophy of the muscle had again, however, not produced any atrophy in the nerve as compared with that of the opposite side (1629a, Table 1).

(3) *Union of muscle nerve with cutaneous nerve*

The sural nerve, lying close to the n.g.m. is a purely cutaneous nerve in the rabbit, supplying no muscles except possibly some unstriped ones in the skin. It contains numerous medullated fibres, none so large as the largest in the n.g.m. Its total diameter is greater than that of the latter and it contains more medullated fibres, but exact counts have not been made.

Union of the n.g.m. with the sural, after crushing high up in the usual way, produced a considerable degree of maturation (Table 3 (*g*)) more in fact than followed direct union of the n.g.m. with its own periphery. Although *D* is greater in this nerve joined with the sural than in the n.g.m. on the opposite side joined with itself (1654j, Table 2 (*a*)) yet the latter contains considerably more fibres above 10μ than does the former. The large *D* of the n.g.m. joined to the sural arises from the presence of numerous fibres of $8–10\mu$ and from reduction in the number under 4μ. The pattern of distribution of the fibre sizes is certainly different from that in nerves united with muscles. The value of *D* is only a very rough indication of the degree of maturation and in this case disguises the fact that union with the muscle produces larger fibres than union with the skin. The latter has, however, evidently produced considerable reduction of small fibres compared with the nerves left without any peripheral connexion (Table 4 (*a*)) and also some increase in the number of fibres in the $8–10\mu$ group.

(4) *Do nerve fibres mature if left without a peripheral connexion?*

The investigations quoted in the introduction show that fibres left unconnected with any peripheral organ remain small. We were interested to pursue this matter further and to find whether there is any progressive increase of diameter and reduction of number under these conditions. Nerves were therefore crushed at a high level and their lower ends cut and turned aside and fixed with plasma to the fascia covering the thigh muscles. In this situation, when the end of the nerve lies on untraumatized connective tissue, it must form a neuroma and can make no connexion with muscle fibres. The animals were then killed after various periods of regeneration, the absence of any connexion with the periphery carefully checked by stimulation and later by sections, and the number and diameter of fibres below the crushed point then determined.

Table 4 (*a*), (*b*) and (*c*) show the results obtained when the nerves had been left for 100, 150 and 200 days. A complication is introduced by the fact that the fibres are smaller and more numerous close to the neuroma than higher up the nerve. The actual levels used for the counts are shown in Table 4 and in the case of rabbit 1543 two pieces of nerve were fixed, allowing counts at two levels. Unfortunately, since the importance of this factor was only discovered during the investigation, the material was not collected in such a way as to allow

Table 4. (*a*), (*b*), (*c*), *fibres regenerated below a crushed point but not allowed to reach their end organs;* (*d*), (*e*), (*f*), *fibres regenerated when nerve sutured after a period of delay*

Nerve	Diameter μ 0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	*N*	*D*	mm. above neuroma
					(*a*) Nerve left 100 days						
1554a		1282	448	244	65	3	0	0	2042	4·08	5
1663c	419	976	164	22	4	0	1	0	1586	3·05	5
1680a	125	1312	710	94	0	0	0	0	2241	3·91	5
1680h	342	1041	399	194	68	9	1	0	2057	4·22	10
1693h	408	1110	568	218	98	19	3	2	2023	4·80	20
								Mean	1990	4·01	
					(*b*) Nerve left 150 days						
1543f	893	964	349	7	1	0	0	0	2214	2·91	15
1543g	108	837	320	158	12	0	0	0	1435	4·12	25
					(*c*) Nerve left 200 days						
1626g	79	742	476	157	20	2	1	0	1477	4·39	10
											Separation mm.
				(*d*) Nerve left unsutured 50 days then joined to nervus plantaris 100 days							
1543c	19	404	201	200	138	57	5	1	1025	6·06	0·5
				(*e*) Nerve left unsutured 100 days then joined to nervus plantaris 100 days							
1554g	141	544	352	251	99	98	26	7	1518	5·85	1·5
				(*f*) Nerve left unsutured 50 days then joined to nervus plantaris 150 days							
1626c	14	224	166	140	91	26	0	0	661	5·97	1·0

counts at exactly the same level to be made in each case, and this affects the validity of the comparisons. Table 5 shows the counts made at three levels above the neuroma of 1680g. The number of fibres decreases steadily passing up the nerve but the decrease is mainly in the smaller fibre groups. The fibres are not appreciably larger higher up in the nerve.

Table 5. *Fibres regenerated at various levels below a crushed point when they are not allowed to return to their end organs*

	Diameter μ										
Nerve	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	*N*	*D*	mm. above neuroma
1680g	597	1804	507	139	17	10	4	0	3078	3·59	5
1680h	342	1041	399	194	68	9	1	0	2054	4·22	10
1680j	123	995	270	167	37	6	1	1	1600	4·20	25

After 100 days the fibres are evidently much smaller and more numerous than in any of the nerves which were allowed to become connected with a periphery. Pl. 2, fig. 6 shows the characteristic appearance, with each of the original Schwann tubes occupied by several small fibres.

After 150 days the appearance at the lower end of the piece is essentially similar to that at the lower ends of the pieces after 100 days. Higher up the fibres are larger than lower down but not larger than at a comparable level after 100 days. We may conclude that there has been no marked change in diameter during the period 100–150 days. The number of fibres is, however, significantly less at this higher level than at any level in any of the nerves at 100 days.

The nerve examined after 200 days confirms these conclusions (Pl. 2, fig. 7). The fibre diameter is not different from that at 100 or 150 days but the number is similar to that after 150 days and less than at 100 days. We may conclude then that fibres do not increase greatly in diameter after the first 100 days of regeneration unless they become connected with the periphery. There may be, however, a slow fall in the number of fibres in such nerves at some distance from the neuroma. In the region close to the end the situation is less certain and there may even be a progressive rise in the fibre number. The reason for the greater number of small fibres in the lower regions of these nerves is not known for certain. It could be that branching is responsible for the increase, but it seems more probable that as suggested by Weiss *et al.* (1945), some of the fibres turn back in the neuroma and grow up into the nerve.

C. *Maturation after delayed union of nerves*

If fibres have regenerated for a while without opportunity to reach the periphery can they later mature if given an opportunity to do so? This question is of considerable practical as well as theoretical interest, since the condition is one often found in nerves damaged over a great length. In three animals we crushed the n.g.m. high up and severed it low down on both sides, turning it

aside in such a way as to prevent any reunion (see previous section). Then after 50 or 100 days the nerve was re-exposed on one side, the neuroma and a portion of the regenerated nerve was removed and the freshened lower end thus obtained was joined to the n. plantaris. The animals were then left for a further 100 days (in one case 150 days) and the nerves removed from both sides. Thus each animal provides us with three stages of regeneration: (1) 50 (or 100) days without peripheral connexion (the pieces removed at the second operation); (ii) 150 (or 200) days without peripheral connexion (the pieces removed from the side operated on once only); (iii) 50 (or 100) days without peripheral connexion, followed by 100 (or 150) days of union with a muscle. The pieces in the first two classes have already been dealt with in the previous section, those of the third class are shown in Table 4 (*d*), (*e*), (*f*). From these it is immediately clear that maturation is possible after these delayed sutures. Thus 1543c, which was united with the periphery shows larger and fewer fibres than 1543f and g from the opposite side which were left ununited. Many fibres of more than 8μ are found, some reaching even 16μ, while the number less than 4μ has fallen markedly. However, the maturation has been less satisfactory than that produced by primary suture of n.g.m. to plantaris (Table 2 (*c*)). The value for D is lower and for N higher in 1543c than in any of the primary sutures. On account of the small number of the latter available the statistical significance of the difference is not quite satisfactory since divergences of this extent would be expected in 5–10 % of cases for D and 2–5 % for N, but the result is highly suggestive. The differences occur in all parts of the distribution, there are fewer large fibres and more small ones after the delayed suture; in fact, it seems that there has been a less rapid growth of the fibres which have made connexion, and with this a less drastic reduction of the surplus.

In 1554g, the nerve sutured after 100 days shows these tendencies even more markedly (Pl. 2, fig. 8). The delayed union has produced maturation; the nerve contains larger and fewer fibres than did the piece removed from it at the second operation, 1554a. But the maturation has been even less satisfactory than in 1543c. The fibres are not very markedly larger and less numerous than in the nerves left unsutured for 100 or 200 days, indeed, 1626g, which was unsutured contains less fibres than 1554g. The effect of the union with the periphery appears most clearly in the presence of large fibres, up to 16μ, there are far more above 8μ than in any of the unsutured nerves, and the distribution compares quite closely in this respect with the upper end of the distributions of simple sutures to n. plantaris (Table 2 (*c*)). But at the lower end there has been a very much less marked reduction in the number of small fibres, as might perhaps be expected if they have become more firmly established. The presence of this large number of small fibres gives an even lower D and a higher N than followed suture after 50 days delay. The probabilities of such large divergences from the means of primary sutures of n. plantaris are 0·05–0·02 for D and $<0{\cdot}01$ for N, and there can be no doubt

that the delayed suture has produced a less complete maturation than the immediate one. Though we may suppose that this difference would be reflected in a less satisfactory functional result we cannot positively assert that this would be so. It is not obvious that the persistence of numerous small fibres exerts any deleterious effect, and of course they might well be removed later.

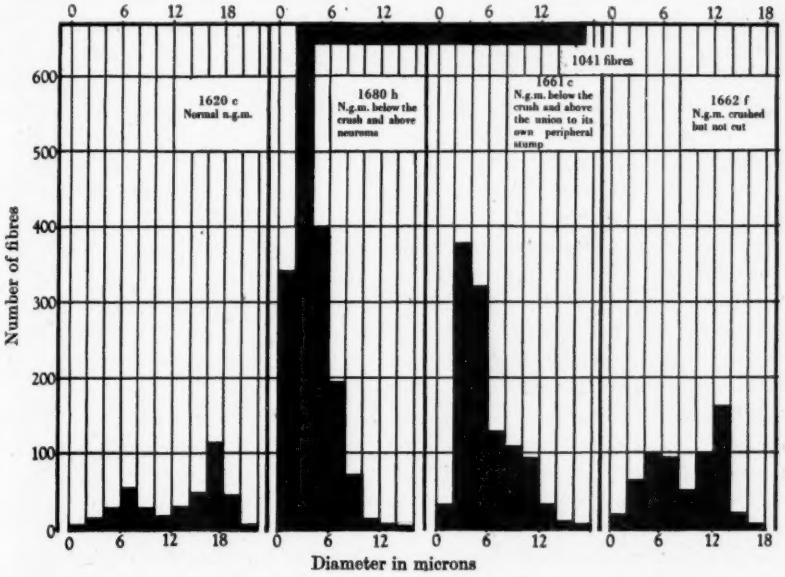
Indeed there is evidence from the one animal left for 150 days after delayed suture that this is so (1626c, Table 4 (*f*)). The number of fibres had here fallen to within the range found 100 days after direct suture of the crushed n.g.m. to n. plantaris and contrasts sharply with that in the unsutured nerve on the opposite side (1626g). However, the fibres have not continued actively to increase in diameter and *D* remains suggestively lower than even 100 days after primary union with plantaris and seems to show that even 50 days of delay in making a suture diminishes the possibility of maturation of any fibres which have been allowed to grow during this period without peripheral connexions.

DISCUSSION

This study of the regeneration of a known number of nerve fibres has fully confirmed the importance of peripheral connexions in controlling the maturation of regenerating nerve fibres. The extent of this influence is so great that recognition of it involves a revolution in our ideas about nerve regeneration. Since the demonstration by Cajal and his school that every new nerve fibre is formed in continuity with an old one we have been inclined to imagine that the influences determining the process of new formation of fibres come mainly from the nerve cell body. It has been recognized that the surroundings of the growing nerve fibre, the Schwann cells and connective tissue tubes also play an important part in the process and Boeke (1935) has especially emphasized the importance of conditions along the regenerating pathway. But it has not been realized that the process of regeneration at any one point along a nerve is not mainly controlled either by the cell body or by the local conditions, but by conditions at distant peripheral points. Weiss (1936, 1941, etc.), has come nearest to this conception in his claim that muscles influence the activity of nerves by conditioning them to conduct messages appropriate to the function of the unit. Whether or not this occurs it is now certain that muscle and other peripheral organs have a very definite influence on the actual structure of the nerve fibres. The growth of a nerve fibre is thus certainly not produced by central factors alone but is under the control of a combination of three sets of influences coming from (1) the cell body, (2) the conditions of the pathway along which the fibre travels, and (3) the peripheral organs with which it becomes connected.

In this present series of experiments the first two factors, the cell bodies and condition of the nerve trunk (including the point of crushing) are alike, yet striking differences in the regenerated nerve fibres are found depending on the

third factor, the peripheral connexions (Text-fig. 1).* When the regenerating stretch ends in a neuroma numerous and small fibres are found, whereas if every fibre is allowed to return to its own ending, examination at the same level after the same time of regeneration shows fewer and larger fibres with a bimodal distribution. There could hardly be a more striking demonstration of the way each nerve fibre is caused to increase to the appropriate diameter by some stimulus coming from the periphery, all superfluous ones being meanwhile removed. If the nerve is cut and sutured between the point of crushing and



Text-fig. 1. Histograms of distributions of fibre sizes in normal nervus gastrocnemii medialis and in the nerve regenerated for 100 days below a crushed point and with various peripheral connexions.

the periphery maturation is much less effective however, and this shows that the peripheral influence is not all powerful. Assuming that the junction is well made we may suppose that some nerve fibres, of the many in the stretch of nerve below the crush, will reach to each muscle fibre or proprioceptor organ. If mere contact with this were able to condition the nerve fibre then it would not matter whether the nerve were cut and rejoined low down or not. Presumably, therefore, the peripheral contacts have full powers of control only over the fibres which possess suitable central connexions. The fact that some

* It should not, however, be forgotten that the blood supply of the nerve is different in the cases with and without peripheral connexion, though there is likely to be an adequate supply along the nerve from above, even in those which end in a neuroma.

maturation can occur in the fibres of a muscle nerve growing into a cutaneous one shows, however, that this limitation is not absolute. The case of nerve 1662f shows that the number of fibres may be so quickly regulated during the regeneration which follows a simple crush that after 100 days little or no excess above normal remains. Weiss *et al.* (1945) found an excess up to 100 % below crushed points even after 1 year of regeneration. There are other indications that their method of crushing was so severe as to disrupt not only the axons but also the Schwann tubes, which would leave a situation approximating to that after nerve suture. The optimum conditions for regeneration are achieved when only the axons are interrupted by a single firm crush between smooth surfaces.

Since after crushing a nerve in this way the regeneration rapidly produces a nerve similar in fibre number and composition to a normal one we must assume that contact with the peripheral organs influences not only the motor but also the proprioceptive sensory fibres. The groups of large fibres (10–20 μ) consist of about one-third sensory fibres, running to the annulo-spiral endings of the muscle spindles (in the cat, Eccles & Sherrington, 1930), and these are presumably re-constituted during regeneration after a simple crush, since the large fibre groups of nerve 1662f contain about the normal number of fibres. Barker & Young (1947) have shown that the innervation of the muscle spindles is very fully regenerated after such operations.

The extent of the powers of regeneration under the influence of the first two factors, the cell body and central stump, and the stretch of nerve distal to the lesion but without an end organ can now be seen more clearly. When axons are interrupted, even if their tubes are left intact they put out a very large number of new branches. In the present work we have counted only those which acquired myelin sheaths and found them sometimes more than five times as numerous as the original fibres of the central stump. We may suppose that there are very many more which do not medullate so that probably at least twenty times the original number of fibres grows down the Schwann tubes below a crushed point. If they make no connexion with the periphery these fibres are none the less able to increase in diameter and to become medullated. Why some grow bigger than others is a problem that would repay investigation. Only very few of them reach more than 10 μ after 100 days and there seems to be no subsequent further growth. The great majority remain as tiny fibres of less than 4 μ, crowding the old Schwann tubes.

The main problems which remain unsettled are the nature of the influence by which the periphery causes increase of diameter and the means by which atrophy of the superfluous fibres is produced. The present series only goes a short way towards giving the answer to the first. Evidently the amount of muscle innervated is an important factor and presumably each nerve fibre that makes contact with muscle fibres receives a stimulus. But is the stimulus proportional to the number of connexions made?

What, if any, is the difference between the stimulus given to efferent and afferent fibres? Apparently contact with any muscle fibre is able to give a stimulus, whether it be in the muscle originally innervated, one with a similar action or an antagonistic muscle. Unfortunately again we cannot yet say whether all muscles produce an equal effect. Nor is there yet sufficient evidence to decide whether the increase of diameter follows when the tip of the fibre comes to rest, setting free for growth purposes energy previously used for agitation along the free surface, as suggested by Weiss *et al.* (1945). The only clue we have to the nature of the action of the muscle is that it is affected by tenotomy (p. 10).

Evidently a muscle which is not able to act when re-innervated cannot give its full effect to the nerve. Yet it should be noted that the maturation is still very much greater in a nerve connected with a tenotomized muscle than in one connected with no periphery at all. We do not yet know whether the tenotomy effect indicates that active functioning is necessary for the maturation of the nerve, or whether it is simply that tenotomy reduces the number of muscle fibres or in some other way influences the power of the muscle to affect the nerve. Since tenotomy alone is not followed by any decrease in nerve fibre diameter, there is no very strong reason to emphasize the importance of effective functioning. In view of the influence of tenotomy on the action of the muscle it is surprising that the increasing atrophy of prolonged denervation does not weaken the power of the muscle to stimulate the maturation of the nerve. Presumably with periods of atrophy longer than 150 days some decline in this power will become evident as the muscle fibres degenerate and disappear (Gutmann & Young, 1944).

The reduction in the excess fibres always goes hand in hand with the increase in size of others under peripheral stimulation, and it is tempting to regard the latter as the cause of the former. The fibres are so tightly packed in the tubes (Pl. 2, figs. 6, 7), that it seems reasonable to suppose that a rapid increase in any one fibre would crush the others and lead to their degeneration. No proof has yet been obtained that this occurs, however, and it is not even certain that the excess fibres do disappear; some at least may remain as unmyelinated fibres. It is even possible that the reduction in number is produced at least in part by union of the fibres within one tube, but there is at present no evidence for this. If the large number of fibres above a neuroma is due to some turning back up the nerve then removal of the neuroma will cause a reduction in number by the degeneration of these recurrent fibres.

Though the results do not enable us fully to establish the nature of the peripheral effect they give every hope that with further development of the method it will be possible to do so. The consistency of the results is sufficiently satisfactory to indicate that a rigid quantitative treatment is now possible. Improvement could be made by further measures of standardization, especially of the breed, age and condition of the animals. The results show that a period of 100 days of regeneration below a crushed point is sufficient to allow very

clear evidence of peripheral influences to emerge. No doubt for some aspects of the problem longer periods will be necessary but these results encourage the attempt to undertake the further analysis of the influence of muscle on nerve on the basis of a 100-day period of regeneration.

SUMMARY

1. The nerve to the medial head of the gastrocnemius of the rabbit is very convenient for quantitative experiments on regeneration since it contains sufficiently few fibres to allow easy counting and runs unbranched for up to 4 cm. It contains about 400 medullated fibres with a bimodal size distribution.

2. The influence of connexion with end organs on regeneration can be examined by crushing the nerve high up, treating the lower end in various ways and then examining the stretch below the crushed point 100 days later. The lesion between cell body and the regenerating region examined is thus kept constant and the very striking differences seen are wholly due to the more peripheral influences acting backwards up the nerve.

3. When the lower end is severed and left in such a way as to prevent reunion with the muscles about 2000 medullated fibres are found in the nerve after 100 days and they are mostly small. After 150 and 200 days the number falls to 1500 but the diameters do not increase. There are more fibres in the lower parts of these nerves, probably because some turn back in the neuroma.

4. If the nerve is crushed high up and severed and sutured low down into its own peripheral stump about 1200 fibres are found after 100 days and some of these are much larger than if the nerve is not allowed to join any end organ.

5. If the nerve is merely crushed high up, without being cut low down, only about 600 fibres are found after 100 days; these are much larger than in the previous cases and show a bimodal distribution.

6. If the lower end of the nerve is joined with the n. plantaris, which is another, larger, branch of the tibial, fewer and somewhat larger fibres are found than after union with its own peripheral stump, only 700 of them after 100 days.

7. Union of the lower end with n. peroneus, which serves antagonistic muscles but is larger than n. gastrocn. med. also gives good maturation, producing 600 fibres, some large.

8. The maturation of the nerve fibres is as rapid when they become united with muscles allowed to atrophy for 100 or 140 days as when they grow direct into freshly denervated muscle.

9. The maturation of the nerve fibres is less rapid when the muscle into which they grow has been left to atrophy after tenotomy.

10. If the nerve is crushed high up and its peripheral end joined to a pure skin nerve (n. suralis) its fibres are reduced in number to 800 after 100 days and they grow in diameter more than if left without a peripheral connexion, but do not reach such large size as when allowed to reach muscles.

11. If the nerve is crushed high up and cut low down so as to be without an end connexion for 50 or 100 days and then later allowed to reach the muscles it is able to mature, but less rapidly than after primary suture. In particular there is delay in removal of the excess of small fibres.

12. The maturation of a regenerating stretch of nerve therefore involves increase in diameter of some of the numerous early fibres and disappearance of others. The two processes go on together and it is probable, though not certain, that the diameter increase causes the reduction in number, the growing fibres perhaps crushing other smaller ones in the same tube and leading to their degeneration.

13. The nature of the influence by which the end organ causes increase of diameter is still not clear. The larger the muscle mass with which a nerve becomes connected, the greater the number of nerve fibres which increase in diameter. Atrophic muscle fibres are able to exert such influence, and union with antagonistic muscle fibres is, at least in the early stages, equally effective. Union with tenotomized muscle produces less rapid maturation but it is not yet clear whether this is because contraction of the neuromuscular unit against resistance is an important factor in its maturation, or whether the effect is produced by changes in the muscle consequent on tenotomy.

14. The growth of a new nerve fibre is thus seen to be under the control not merely of central influences, but of three sets of factors: (1) the cell body and central stump, (2) the condition of the pathway along which the fibre travels, and (3) the connexions which it makes with an end organ.

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REFERENCES

Barker, D. & Young, J. Z. (1947). *Lancet* (in the Press).

Boeke, J. (1935). Nervenregeneration. In Bumke, O. & Foerster, O. *Handbuch der Neurologie*, **1**, 995–1122.

Duncan, D. (1934). A relation between axone diameter and myelination determined by measurement of myelinated spinal root fibres. *J. comp. Neurol.* **60**, 437–462.

Eccles, J. C. & Sherrington, C. S. (1930). Numbers and contraction values of individual motor-units examined in some muscles of the limb. *Proc. roy. Soc.* B, **106**, 326–356.

Fisher, R. A. (1928). *Statistical Methods for Research Workers.* Edinburgh: Oliver and Boyd.

Gutmann, E., Guttman, L., Medawar, P. B. & Young, J. Z. (1942). The rate of regeneration of nerve. *J. exp. Biol.* **19**, 14–44.

Gutmann, E. & Sanders, F. (1943). Recovery of fibre numbers and diameters in the regeneration of peripheral nerves. *J. Physiol.* **101**, 489–518.

Gutmann, E. & Young, J. Z. (1944). Reinnervation of muscles after various periods of atrophy. *J. Anat., Lond.*, **78**, 15–43.

Hammond, W. S. & Hinsey, J. C. (1945). Diameters of nerve fibres in normal and regenerating nerves. *J. comp. Neurol.* **83**, 79–91.

Sanders, F. K. & Young, J. Z. (1944). The role of the peripheral stump in the control of fibre diameter in regenerating nerves. *J. Physiol.* **103**, 119–136.

Sanders, F. K. & Young, J. Z. (1945). Effect of peripheral connexion on diameter of nerve fibres. *Nature, Lond.*, **155**, 237–238.

Sanders, F. K. & Young, J. Z. (1946). The influence of peripheral connexion on the diameter of regenerating nerve fibres. *J. exp. Biol.* **22**, 203–212.

Simpson, S. A. & Young, J. Z. (1945). Regeneration of fibre diameter after cross-union of visceral and somatic nerves. *J. Anat., Lond.* **79**, 48–64.

Weiss, P. (1936). Selectivity controlling the central peripheral relations of the nervous system. *Biol. Rev.* **11**, 494–531.

Weiss, P. (1941). Self-differentiation of the basic patterns of coordination. *Comp. Psychol. Monogr.* **17**, no. 4.

Weiss, P., Edds, MacV. & Cavanaugh, M. (1945). The effect of terminal connexions on the caliber of nerve fibres. *Anat. Rec.* **92**, 215–233.

Weiss, P. & Taylor, A. C. (1944). Further experimental evidence against 'neurotropism' in nerve regeneration. *J. exp. Zool.* **95**, 233–257.

Young, J. Z. (1935). Osmotic pressure of fixing solutions. *Nature, Lond.* **135**, 823–824.

EXPLANATION OF PLATES

All figures are of preparations fixed with Flemming's fluid and stained with Weigert's method. The transverse sections are at a magnification of 125 ×, the longitudinal sections of 12·5 ×.

Plate 1

Fig. 1. Normal nervus gastrocnemii medialis of rabbit (1620d).

Fig. 2. Level at which nerve had been crushed 100 days previously (1654f).

Fig. 3. Level at which nerve had been cut and joined with plasma to its own peripheral stump 100 days previously (1654d).

Fig. 4. Nervus gastrocnemii medialis below crushed point, above suture to own peripheral stump. 100 days (1647b).

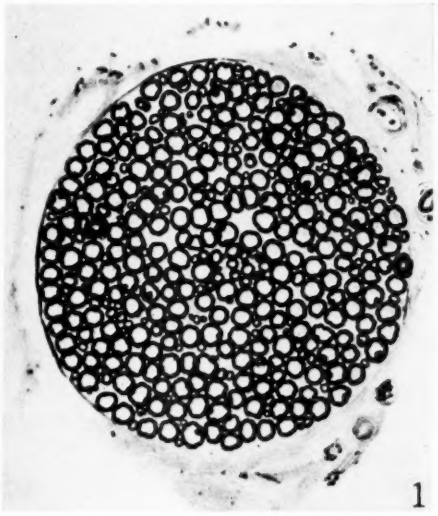
Plate 2

Fig. 5. Nervus gastrocnemii medialis below point crushed 100 days previously. No severance or suture (1662f).

Fig. 6. Nervus gastrocnemii medialis below crushed point, above neuroma. No union with periphery. 100 days (1680h).

Fig. 7. As Fig. 6. 200 days (1626g).

Fig. 8. Nervus gastrocnemii medialis crushed and left to regenerate without union with periphery for 100 days, then joined to nervus plantaris for 100 days. The fibres have become large and few (1554g).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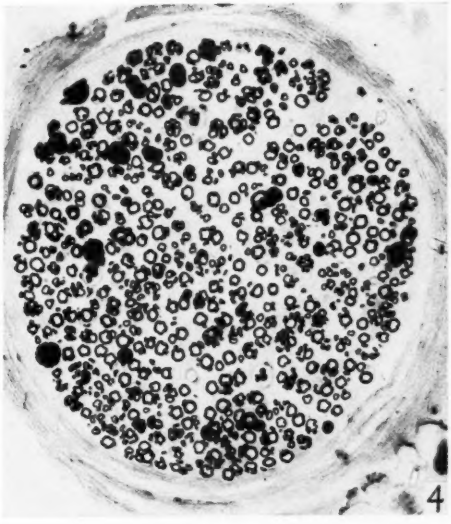
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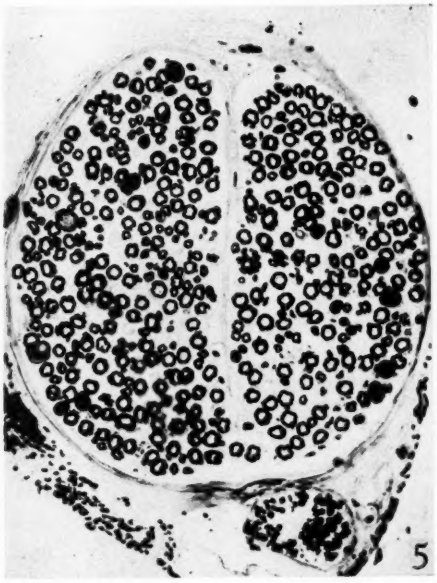

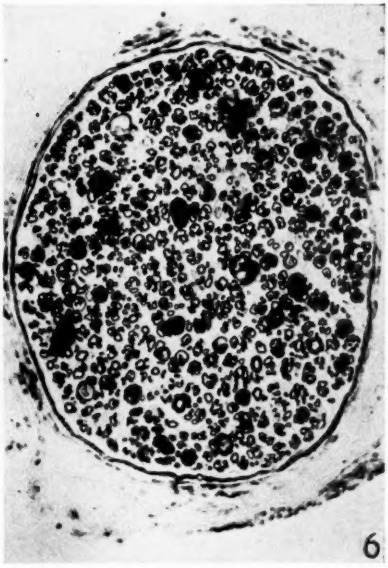

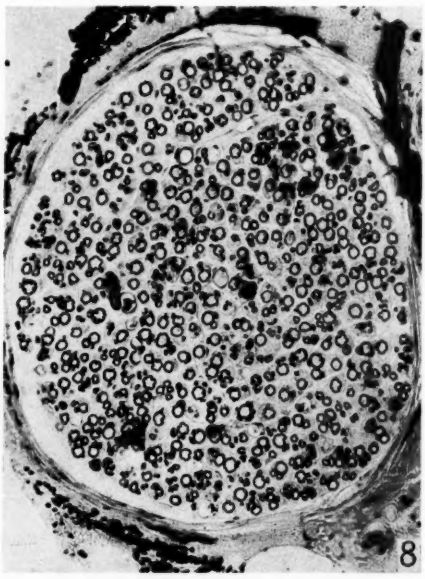

AITKEN, SHARMAN AND YOUNG—MATURATION OF REGENERATING NERVE FIBRES

SENSORY FIBRES IN THE HYPOGLOSSAL NERVE

By A. A. TARKHAN and I. ABOU-EL-NAGA

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It has been found (Tarkhan, 1936*a*) that in the rabbit section of the hypoglossal nerve results in degeneration of all nerve endings in the muscles of the tongue, and that section of the anterior rami of the upper four cervical nerves causes no degeneration either in the fibres of the hypoglossal nerve or in its branches in the tongue. In another publication (1936*b*) the same author recorded a rise in blood pressure in the cat after stimulation of the central end of the hypoglossal nerve and concluded that this nerve is sensori-motor. This rise in blood pressure is not affected by extirpation of the superior cervical sympathetic ganglion. It was found also that stimulation of the central end of either the lingual or the hypoglossal nerve resulted in reflex movements of the tongue, provided the other hypoglossal nerve was intact. It was therefore concluded that the central afferent path of the lingual nerve is connected with the hypoglossal nuclei and that the sensory path of the hypoglossal nerve, contrary to its motor path, is partially crossed in the medulla oblongata.

Downman (1939) reported that electrical stimulation of the central end of the hypoglossal nerve in the decerebrate cat caused dilatation of the pupil, rise of blood pressure, and general muscular movement. He still obtained dilatation of the pupil even after bilateral removal of the upper two cervical spinal ganglia and section of the spinal cord below the second cervical vertebra. These reflex movements could no longer be obtained, however, after section of the vago-accessory-hypoglossal group of nerves close to the skull; in certain experiments the reflexes were abolished by section of the vagus and accessory nerves alone. Downman thereupon assumed that in some animals the afferent fibres of the hypoglossal trunk enter the skull with the vago-accessory group, while in others they enter with the hypoglossal fibres. He recorded, however, that removal of the ganglion nodosum did not modify the reflexes aroused by hypoglossal stimulation.

Weddell, Harpman, Lambley & Young (1940) obtained dilatation of the pupil by stimulation of the intact hypoglossal nerve in the rat. This reflex was abolished by removing the superior cervical sympathetic ganglion. The authors concluded that dilatation of the pupil was due to stimulation of the sympathetic fibres in the hypoglossal nerve.

Boyd (1941), studying the hypoglossal nerve in the rabbit, concluded that any afferent fibres in this nerve could reach the central nervous system only

by way of the upper cervical dorsal roots. He also stated that no nerve connexion between the hypoglossal nerve and the ganglion nodosum could be demonstrated in his preparations.

The present investigation has been carried out with the purpose of elucidating further the course and origin of the afferent component of the hypoglossal nerve.

METHODS AND MATERIAL

Our material was obtained entirely from dogs. Three series of experiments were performed.

In the first series, the rootlets of the hypoglossal nerve were divided intracranially, and the degenerative changes occurring in the trunk of the nerve peripheral to the point of division were studied.

In the second series, the superior cervical sympathetic ganglion, the upper cervical spinal ganglia, or the ganglion nodosum were removed and any consequent degeneration in the hypoglossal nerve observed.

In the third series, we studied the effect of stimulation of the central end of the divided hypoglossal nerve on the blood pressure in normal animals and in animals treated as were those of the first and second series.

SERIES I

Intracranial division of the hypoglossal nerve

Operation. Dogs are anaesthetized by an intravenous injection of chloralose in doses of 65–90 mg./kg. body weight. To facilitate operative manipulations, shrinkage of the brain is first brought out by an injection of 10–30 c.c. of 30 % hypertonic saline solution. Both common carotids are then temporarily occluded by bulldog forceps to minimize the amount of bleeding during the operation. After some experience the manipulations can be carried out with greater facility and the last two measures may be dispensed with.

A midline incision is made extending from the external occipital protuberance to the spine of the third cervical vertebra. The median raphe is longitudinally divided, the neck muscles retracted laterally and the suboccipital membrane carefully divided. The dura mater is punctured to allow gradual escape of cerebrospinal fluid and, finally, a paramedian incision exposes the medulla. The latter is gently displaced until the fan-like hypoglossal rootlets become apparent, and with an iris knife these are divided. The field is cleaned and the divided structures sutured. When the animal recovers from anaesthesia and begins to lap water, the tongue is found to be deviated towards the operated side if the rootlets have been successfully divided. Further confirmation is obtained by careful dissection of the site of the operation after death.

The hypoglossal nerve was cut intracranially in five dogs. The animals were allowed to live 33–134 days after the operation. After death the hypoglossal nerve and the vagus, accessory and glossopharyngeal nerves were

dissected out. This material was fixed, embedded in paraffin, serially sectioned longitudinally and then stained with silver. The silver technique adopted was in most cases that described by Tarkhan (1934); in a few instances, the Gros-Bielschowsky method was employed instead.

Results

Exp. I. Dog killed 33 days after the operation. Most of the fibres of the hypoglossal nerve were completely degenerated and had been absorbed. Some healthy nerve fibres, however, were seen in the middle of the degenerated mass. They were noticeable in the trunk of the nerve both above and below the origin of the descendens hypoglossi. In addition, there were some separate bundles of healthy thick fibres in the part of the nerve proximal to the point of origin of its descending branch. Most of these bundles were found to direct themselves into the descendens hypoglossi. A connexion which, during dissection, was seen to exist between the vagus and the hypoglossal nerves was found on histological examination to be completely fibrous; but another filament which reached the hypoglossal nerve from the vertebral column consisted mainly of nerve fibres. These fibres seemed to run separately along the side of the nerve and in all probability are the same fibres which travelled into the descending branch.

On the proximal part of the hypoglossal nerve, a typical unipolar cell with a glomerulus and an epithelioid capsule was observed (Pl. 1, fig. 1). A collection of nerve cells, i.e. a nerve ganglion, was also seen along the course of the descending branch of the hypoglossal nerve, just after it had left the trunk of the main nerve (Pl. 1, fig. 2). The ganglion was found to be connected by a bundle of nerve fibres with the distal portion of the hypoglossal nerve.

Exp. II. Dog killed 42 days after operation. Study of the degenerated nerve showed the presence of both thick and thin healthy fibres dispersed among completely degenerated ones, this picture being present throughout the whole nerve, including all its lingual branches. No nervous connexion could be demonstrated between the hypoglossal and vagus or accessory nerves. A bundle of healthy fibres, most probably spinal in origin, proceeded separately on the lower margin of the nerve and continued into the descendens hypoglossi. The descendens hypoglossi was seen during dissection to receive a similar, but totally separate, bundle directly from the spinal nerves.

About fifteen ganglion cells were found to be distributed either singly or in groups along the whole length of the cervical course of the nerve. They were either unipolar or bipolar (Pl. 1, fig. 3) and extended as far as the entrance of the nerve into the muscles of the tongue.

Exp. III. Animal killed 61 days after operation. Healthy fibres were observed as in the previous experiments (Pl. 1, fig. 4). A bundle of fibres came to the nerve from the first and second cervical nerves, and continued into the descendens hypoglossi.

More than ten ganglionic cells were seen along the trunk of the nerve. Some

cells formed a group at the origin of the descendens hypoglossi. No connexion was seen between the hypoglossal nerve and the other cranial nerves.

Exp. IV. Animal killed 134 days after operation. Healthy fibres were demonstrated in the degenerated nerve and only two cells were seen.

Exp. V. Animal killed 55 days after operation. The healthy fibres observed in the previous experiments were demonstrable both in the trunk and in the branches of the nerve. All fibres in the descendens hypoglossi were completely healthy. No nerve cells were seen along the course of the hypoglossal nerve.

SERIES II

A. *Removal of the superior cervical sympathetic ganglion with or without previous section of the intracranial rootlets of the hypoglossal nerve*

(1) *Without section of the XII n. rootlets.* This operation was performed on two dogs, one of which was killed after 5 days and the other after 14 days. The hypoglossal nerve on the side of the operation was dissected out, serially sectioned longitudinally and stained with silver as in series I. Examination of the hypoglossal nerve distal to the origin of the descendens hypoglossi showed that its component fibres in both cases were completely healthy.

(2) *With section of the XII n. rootlets.* The same operation was performed on two dogs in which the hypoglossal rootlets had been cut intracranially 1 week previously. The animals were killed 55 days after the second operation, and the material was prepared and examined as before.

Examination of the hypoglossal nerve in both animals showed the presence of completely healthy fibres, mainly of small calibre, in the trunk of the degenerated nerve. These could not be due to regeneration, since it was our invariable practice in all dissections to verify the absence of any connexion across the site of division of the nerve.

B. *Removal of the upper cervical spinal ganglia*

In the dog the first spinal ganglion is a minute body located in a tunnel in the posterior arch of the atlas. The second ganglion is easily found outside the vertebral column; the third lies hidden underneath the articular process of the vertebra above.

In two dogs these three ganglia were removed on one side. One of the dogs was killed after 6 days and the other after 15 days.

Neither by silver in the first dog, nor by the Marchi technique in the second could any degeneration be detected in the trunk or branches of the hypoglossal nerve.

C. *Removal of the ganglion nodosum*

Since the jugular ganglion lies deeply buried in the bones of the skull, we had to content ourselves with the removal of the ganglion nodosum. The operation was performed on four dogs. In the first two experiments, the central stump of the vagus nerve was avulsed with the hope of removing or

injuring the jugular ganglion, while in the third and fourth experiments avulsion was avoided for fear of injuring the trunk of the hypoglossal nerve. The dogs were killed after 5 days, and the hypoglossal nerve was then prepared for microscopic examination.

In the first two dogs the hypoglossal nerve on the operated side was found to contain a certain proportion of degenerating fibres, approximately 5 % of the total in each section of the nerve. An accurate count in longitudinal sections, however, is misleading; while in transverse sections stained with silver after the lapse of this short period such a count is hardly justifiable, since many of the degenerating fibres may appear healthy. In the third and fourth experiments—where no avulsion was performed—nearly the same amount of degeneration was found, but was much less in the case of the fourth experiment, where ganglionic cells happened to be present on the trunk of the hypoglossal nerve at the origin of the descendens hypoglossi. Most probably these ganglionic cells gave origin to the greater part of the sensory fibres supplying the muscles of the tongue in this animal.

SERIES III

Blood-pressure experiments

Animals were anaesthetized with chloralose. The hypoglossal nerve, on one side, was exposed in the neck and freed from the surrounding tissue by careful dissection, from the base of the skull distally towards the tongue. The trunk of the nerve was ligatured peripherally and cut distal to the ligature. All possible connexions with the central part were severed, and in this manner a long stretch of the central end of the nerve was prepared. This was stimulated mechanically or by a faradic current, care being taken to avoid diffusion of the current to adjacent structures. The arterial blood pressure was recorded from the femoral artery.

Results. In a normal dog, mechanical stimulation of the hypoglossal nerve produced a rise of blood pressure of about 22 mm. Hg, while electrical stimulation produced a rise of about 10 mm. Hg.

In another animal (Pl. 1, fig. 5) either mechanical or electrical stimulation produced an initial rise of 12 mm. Hg, followed by a steep fall. Stimulation of the central end of the cut sciatic nerve produced similar results.

The same procedures were repeated in two dogs 5–6 days after intracranial section of their hypoglossal rootlets. Electrical stimulation of the central end of the hypoglossal nerve in these two dogs on the operated side produced a rise of blood pressure of about 20 mm. Hg. Then the ganglion nodosum was removed in both animals and the stimulation of the nerve repeated. No rise of blood pressure was produced in the first animal and only a slight rise in the second.

DISCUSSION

Langworthy (1924) was the first to report that section of the hypoglossal nerve leads to complete degeneration of both motor and sensory endings in the muscles of the tongue. The nervus hypoglossus was therefore assumed to convey proprioceptive fibres from these muscles. The same results were obtained by Tarkhan (1936*a*), who confirmed that this nerve is sensori-motor. It was therefore expected that section of the efferent fibres only of this nerve would leave a certain amount of unaffected healthy fibres in the mixed trunk. In the present investigation, the hypoglossal rootlets were divided intracranially; the examination of the degenerated nerve always showed the presence of a certain number of intact fibres. Such intact fibres, which must have joined the nerve extracranially, may be either afferent or efferent, and if the latter can come from the sympathetic chain only.

Langworthy (1924), 30 days after extracranial section of the hypoglossal nerve in the cat, observed within the tongue 'fibres of small calibre which apparently followed the arteries' (p. 280). Weddell *et al.* (1940), in rats killed 5 days after section of the hypoglossal nerve at the base of the skull, found undegenerated sympathetic fibres of hypoglossal origin passing to the blood vessels. Boyd (1941), in the rabbit, traced fibres from the superior sympathetic ganglion to the hypoglossal trunk and suggested that they were sympathetic fibres proceeding to the lingual vessels. Such communicating fibres, however, were quite inconstant, so he suggested 'that a number of the sympathetic fibres reach the lingual blood vessels along the alternative route of the lingual artery'. From this it appears that no definite evidence has been put forward to support the presence of sympathetic fibres in the trunk of the hypoglossal nerve.

We removed the superior cervical sympathetic ganglion in two dogs which were then killed, 5 and 15 days after the operation. No degeneration whatsoever was detected by the silver technique in the trunk of the hypoglossal nerve. Furthermore, removal of the superior sympathetic ganglion after section of the hypoglossal rootlets left unaffected, after the lapse of 55 days, some fibres in the trunk of the nerve which therefore are not of sympathetic origin.

That the intact fibres, found in the trunk of the hypoglossal nerve after section of its rootlets, are afferent fibres finds support in the work of Tarkhan (1936*b*), Acheson, Partington & Rosenblueth (1936) and Downman (1939), all of whom recorded a rise of blood pressure in the cat and dog on central stimulation of the hypoglossal nerve. The first author also recorded that such stimulation provoked reflex movements of the tongue provided the other hypoglossal nerve was intact. Such reflex movements, or such a rise in blood pressure, must, therefore, be mediated through afferent fibres in the central end of the cut hypoglossal. Downman (1939), in the decerebrate cat, reported reflex dilatation of the pupil on central stimulation of the cut hypoglossal.

Weddell *et al.* (1940) obtained similar results from stimulation of the intact nerve in the rat, but they also claim that such reflex action disappears on removal of the superior cervical sympathetic ganglion.

They expressed their doubts, however, as to the validity of accepting such a rise of blood pressure, dilatation of the pupil and contraction of the nictitating membrane as indicative of the presence of afferent impulses in such a nerve. Such doubts, though perhaps justifiable when the intact nerve is stimulated, as was their practice, are groundless when the central cut end is stimulated. Any rise of blood pressure, as was obtained with such stimulation in our experiments, can only be explained by the presence of afferent fibres in the trunk of the stimulated nerve.

In this investigation we obtained in dogs a rise of blood pressure after central stimulation of the divided hypoglossal nerve. A similar effect was recorded in animals in which the intracranial rootlets had previously been cut. This seems to prove that afferent fibres in the hypoglossal trunk do not accompany its efferent fibres into the brain.

Concerning the place of origin of these afferent fibres, three possibilities present themselves: the upper cervical spinal ganglia, the ganglion cells on the hypoglossal nerve or the ganglia associated with the neighbouring cranial nerves.

Much controversy has raged over the first possibility. Langworthy (1924), in one cat, claimed that avulsion of the hypoglossal nerve resulted in chromatolysis of the cells of the second cervical spinal ganglion. One such observation, however, cannot be conclusive. Van der Sprenkel (1924) speaks of connexions between the second cervical nerve and the descendens hypoglossi in serial sections of hedgehog embryos. This author suggests that afferent fibres from the second cervical ganglion pass into the descendens hypoglossi and continue distally into the hypoglossal nerve, but no experimental evidence was adduced in favour of this suggestion. Corbin, Lhamon & Petit (1937), found in the rhesus monkey, that removal of the second cervical ganglion caused degeneration of about 1 % of the fibres of the hypoglossal nerve. On the other hand, Hinsey & Corbin (1934) found no degeneration in the trunk of the hypoglossal nerve after removal of the anterior rami of the second, third and fourth cervical nerves.

We removed the upper three cervical ganglia in the dog and obtained no degeneration whatsoever in the trunk of the hypoglossal nerve.

The silver technique we employed has been constantly reliable. We are, therefore, certain that, at least in the dog, the upper cervical ganglia contribute no fibres to the hypoglossal nerve. Those who speak of such a possibility report a small percentage of degeneration in the hypoglossal nerve after the removal of the upper cervical ganglia. Such a percentage, even if proven, is so meagre that it can account for only a very small proportion of the afferent fibres from the tongue.

As to the question of the presence of ganglion cells along the course of the hypoglossal nerve, the data obtained by the different investigators seem to be

conflicting. From the work of Tarkhan (1934), from the present investigation and from some recent data obtained in this department, we feel justified in concluding that sensory cells, few or many, singly or in groups, do exist in some cases along the course of this nerve; their occurrence, however, is inconstant. The negative findings reported by both Boyd (1937, 1941) and Carleton (1938) may be ascribed to their different methods of approach. A discussion of the differences between our respective methods, together with a report on new data already referred to above, will be included in a subsequent communication to this *Journal*.

Pearson's reports (1943, 1945) on the presence of sensory cells on the intramedullary course of the hypoglossal nerve in man are in support of the mixed nature of this nerve, and may explain in part why sensory cells are such an inconstant finding on the extramedullary part of this nerve.

Finally, there remains the third possibility: that the afferent fibres in the hypoglossal nerve may arise from cells incorporated within ganglia present on the neighbouring cranial nerves. Langworthy (1924), after avulsion of the hypoglossal nerve in the cat, described chromatolysis in the cells of the ganglion nodosum. Downman (1939), on the other hand, reported that the reflex responses he obtained on central stimulation of the hypoglossal were abolished in certain cats by section of the vagus-accessory group but not by removal of the ganglion nodosum. Boyd (1941), in the rabbit, found no connexion between the ganglion nodosum and the hypoglossal nerve.

In the present investigation we removed the ganglion nodosum in four dogs. In three of them, degenerated fibres were easily demonstrable in the trunk of the hypoglossal nerve. In the fourth experiment, where a ganglion was found on the trunk of the hypoglossal nerve, the number of degenerated fibres was greatly diminished. It is therefore justifiable to conclude that the ganglionic cells on both hypoglossal and vagus nerves share in supplying the afferent fibres in the former nerve; the number of fibres originating from either source varies from one animal to another.

As to the path by which these afferents enter the central nervous system, Downman (1939) states that though all the fibres enter in the bulbar region, the exact path they take into the cranium appears to vary. In cats they enter either through the jugular foramen with the vagal fibres or through the hypoglossal canal with the hypoglossal nerve. He added that it seems very probable that the fibres entering through the hypoglossal canal communicate later with the vagal fibres.

We have stimulated the central end of the hypoglossal nerve in two dogs, after intracranial section of its rootlets, and obtained a rise of blood pressure nearly as high as the rise we obtained from normal dogs. Then the ganglion nodosum was removed in both animals; subsequent stimulation of the central cut end of the hypoglossal nerve still produced a rise of blood pressure in one animal but not in the second. Possibly, in the first animal a more centrally placed connexion existed between the hypoglossal and vagus nerves.

SUMMARY

1. Stimulation of the central cut end of the hypoglossal nerve in dogs causes a rise of blood pressure. This may be taken as evidence that this nerve is mixed.

2. Section of the hypoglossal rootlets, in the dog, leaves unaffected a certain number of myelinated fibres. These presumably constitute the afferent component of this nerve. In the trunk of the nerve the number of afferent as compared to efferent fibres is proportionately smaller than in other predominantly motor nerves.

3. Removal of the upper three cervical spinal ganglia in the dog results in no degeneration in the fibres of the hypoglossal nerve.

4. Removal of the superior cervical sympathetic ganglion in the dog results in no degeneration in the fibres of the hypoglossal nerve. Thinly myelinated fibres seen in the trunk of the nerve after the section of its rootlets are not of sympathetic origin, since they persist after removal of the superior cervical sympathetic ganglion.

5. The ganglion nodosum contributes a certain amount of fibres to the hypoglossal nerve. The number of these fibres seems to be inversely proportional to the number of ganglionic cells on the hypoglossal nerve.

6. Sensory cells on the trunk of the hypoglossal nerve are frequently present in the dog. They occur both singly and in ganglionic collections.

7. It is suggested that peripherally these afferent fibres run entirely through the hypoglossal nerve, while intracranially they enter the brain mostly with the vagal fibres.

REFERENCES

ACHESON, G. H., PARTINGTON, P. F. & ROSENBLUETH, A. (1936). *Amer. J. Physiol.* **115**, 308.
BOYD, J. D. (1937). *J. Anat., Lond.*, **73**, 147.
BOYD, J. D. (1941). *J. Anat., Lond.*, **75**, 330.
CARLETON, A. (1938). *J. Anat., Lond.*, **72**, 502.
CORBIN, K. B., LHAMON, W. T. & PETIT, D. W. (1937). *J. comp. Neurol.* **66**, 405.
DOWNMAN, C. B. B. (1939). *J. Anat., Lond.*, **73**, 387.
HINSEY, J. C. & CORBIN, K. B. (1934). *J. comp. Neurol.* **60**, 37.
LANGWORTHY, O. R. (1924). *J. comp. Neurol.* **36**, 273.
PEARSON, A. A. (1943). *Biol. Abstr.* **17**, 1699.
PEARSON, A. A. (1945). *Biol. Abstr.* **19**, 1158.
SPRENKEL VAN DER, H. R. (1924). *J. comp. Neurol.* **36**, 219.
TARKHAN, A. A. (1934). *J. Anat., Lond.*, **68**, 293.
TARKHAN, A. A. (1936*a*). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **105**, 349.
TARKHAN, A. A. (1936*b*). *Arch. Psychiat. Nervenkr.* **105**, 475.
WEDDELL, H., HARPMAN, J. A., LAMBLEY, D. G. & YOUNG, L. (1940). *J. Anat., Lond.*, **74**, 255.

EXPLANATION OF PLATE

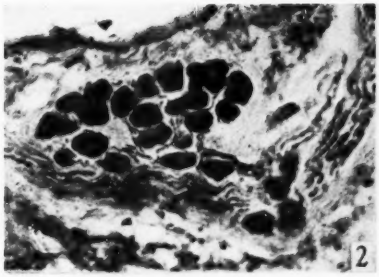
Fig. 1. Microphotograph of a typical unipolar cell seen on the trunk of a degenerated hypoglossal nerve the rootlets of which were cut 33 days before killing the animal. Exp. I. ×200.

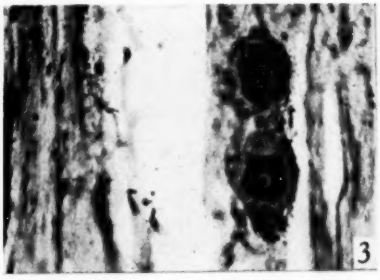
Fig. 2. Microphotograph of a longitudinal section of the hypoglossal nerve at the origin of the descendens hypoglossi. A sensory ganglion is seen in the section. Fibres from this ganglion were traced in serial sections and found to run distally in the hypoglossal nerve. Exp. I. ×200.

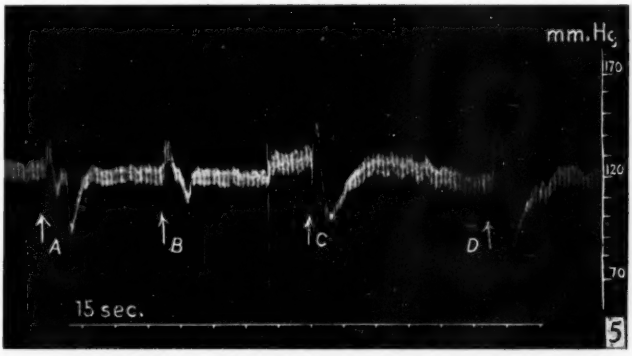
Fig. 3. Microphotograph showing the presence of two ganglionic cells along the course of a degenerated hypoglossal nerve. Exp. III. ×200.

Fig. 4. Microphotographs from a longitudinal section of the hypoglossal nerve 61 days after section of its rootlets. Healthy nerve fibres can easily be seen in between the degenerated ones. Exp. III. ×200.

Fig. 5. A, rise of blood pressure succeeded by a steep fall after applying a ligature to the left hypoglossal nerve in a chloralosed dog; B, a similar effect obtained on faradic stimulation of the same nerve central to the ligature; C, after ligature of the right sciatic nerve; and D, after faradic stimulation of the right sciatic nerve. Blood pressure recorded from left femoral artery.







TARKHAN AND ABOU-EL-NAGA—SENSORY FIBRES IN THE HYPOGLOSSAL NERVE

THE DEVELOPMENT OF JOINTS

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INTRODUCTION

Since the classical work of Bernays (1878), Schulin (1879) and Hagen-Torn (1882), the general course of the development of joints has been well understood, and the work of Lewis (1902) and Bardeen (1905) has extended our knowledge by the description of carefully chosen series of human embryos. Many special studies bearing on the development of joints, some directly and some indirectly, have been published since, and the more important of these will be mentioned in the appropriate places in the text. But no general work based on an adequate series of preparations taken from the larger joints of man has appeared for many years, and it may be useful to present a simple account of the course of development, even if it involves the examination of stages already described and figured elsewhere.

Recently, moreover, Walmsley (1940) and Whillis (1940) have given new accounts of the development of joints, somewhat at variance with those previously received. Walmsley found that the patella and femur, though originally developed quite separately, later became fused by their perichondria, and considered that this fusion played an essential part in the formation of the articular surfaces. Whillis found that in relatively late embryos, at stages long after the peripheral parts of the joint cavities had appeared, adjacent articular cartilages were joined together by a stratum of intervening cells, which eventually disappeared leaving the cartilages quite continuous with each other. Some of the specimens considered in this paper bear directly on the questions raised by these authors.

The series of embryos available covers the period from just before the first appearance of the joint rudiments at 10 mm. to the appearance of the cavities at 30–34 mm. with no serious gaps. Thereafter material is scanty, so that only a few remarks on later development can be offered and there is no discussion on the changing relationship of the articular surfaces and capsules in the late embryo and infant, a subject reviewed by Schulin (1879), nor of the various mechanical theories put forward to account for the development of the shapes of the articular surfaces, fully discussed by Hesser (1926).

INTERZONES OF THE LARGER JOINTS, 11–14 mm.

The skeletal blastema appears in the fore-limb bud as a diffuse condensation of the mesenchyme at 7 mm., and is complete as far as the hand plate at 9 mm., while that of the hind-limb bud appears a little later, but is distinct by 9 mm.

(reconstructions of Lewis, 1902, and Bardeen, 1905, embryos II, CLXIII). At 10 mm. (Fig. 1*) just before the first chondrifications become recognizable, the skeletal blastema is more clearly marked off from the surrounding tissues, but is still continuous in some places with the pre-muscle masses. The blastema of the upper arm is marked off from the extensor mass, but between it and the median nerve there is as yet no sign of differentiation of the intervening condensed mesenchyme. The blastema as a whole has the general form of the future skeleton (as shown in Lewis's and Bardeen's reconstructions), but though the general positions where the scapula and the long bones will appear later can already be distinguished, there is no sign of any morphological differentiation at these sites. The hind-limb of this embryo shows a similar structure, but the tissues are less well differentiated.

In a slightly older embryo (10·5 mm., Fig. 2) the blastema is not so easy to distinguish from the surrounding tissues, for the muscle masses of the upper arm have become very dense and those of the lower arm have now differentiated and are continuous with the blastema. In the hind-limb of this embryo (Fig. 3) the muscles are not yet differentiated in the distal segments, so the tibio-fibular part of the blastema and the foot plate stand out clearly from the neighbouring mesenchyme, while the femoral region is already obscured by the developing muscles, so that the structure resembles that of the fore-limb in the 10 mm. embryo. These specimens illustrate the late blastemal phase before the joints have appeared, but Murray (1926) and Fell & Canti (1934) have shown that in the chick at a similar stage the blastema is already determined as to the organs it is to form, though still morphologically undifferentiated.

The skeletal elements of the girdles and the larger bones appear as centres of chondrification in the interior of the blastema, one for each element at 11 mm. These were seen by Lewis (1902, embryo CIX), who stated that the centres appeared at about the same time as the mesenchymal condensations for the main muscle masses of the limbs, but the two embryos already described show that the muscle condensations precede the chondrifications. Each cartilaginous element is completely surrounded by blastemal tissues and is expanding by further chondrifications of these tissues, that is by appositional growth (Retterer, 1902). So the unchondrified blastema remaining between the cartilages gradually becomes thinned to form a series of plates, the interzones (Bruch, 1851, 'Zwischenmasse'; Henke & Reyher, 1874, and Schulin, 1879, 'Zwischenzone'; Whillis, 1940, and Walmsley, 1940, 'articular discs'), and it is these plates that form the first morphological indication of the joints.

By 12 mm. the cartilages at the shoulder and elbow (Fig. 4) are already adopting their characteristic shapes and the interzones, though still relatively thick, are quite distinct, each formed by a mass of undifferentiated blastemal tissue between the cartilages. At the knee (Fig. 5) the interzone is as yet

* All figures are shown on Plates 1–4, the figures being numbered consecutively.

indistinct, for the condylar region of the femur is still in the earliest ('prochondral') stage of chondrification, and the centre for the tibia is still very small. At the hip the pelvis is still represented by dense blastemal tissue continuous with that forming the upper end of the femur, and the interzone has not yet made its appearance. In Bardeen's (1905) embryo CIX of 11 mm. chondrification of the hind-limb was more advanced than in my 12 mm. specimen or Petersen's (1893) 'S' of 12·6 mm., for the centres for the pubis and ischium had already appeared. But no doubt there is great individual variation in development.

At 13 mm. the chondrification is more advanced and the skeletal elements have acquired some of their characteristic trochanters, condyles, etc., while many of the muscles can now be identified individually and have been reconstructed (Lewis, 1902, embryo CLXXV). At the shoulder (Fig. 7), elbow (Fig. 8), hip and knee (Fig. 10), each interzone is formed by a distinct plate of condensed tissue whose cells pass without interruption into the cartilages on either side. Peripherally the interzone is continuous with the perichondrium of the skeletal elements, but while at the knee the perichondrium is demarcated from the neighbouring condensed tissues, particularly of the quadriceps tendon, by a layer of loose connective tissue, at the shoulder and hip the interzones, perichondria and condensed tissues are in unbroken continuity. Within the interzone there is no sign of differentiation; at the hip it is a 'dense mass of sclero-blastema' at 13 mm. (Lewis, 1902, embryo CLXXV), in other joints a 'solid mass of mesenchyme', and it remains for some time in this state while the other tissues of the joint develop round it.

At 13 mm. the interzones show little sign of the shape of the future articular surfaces. At the shoulder the head of the humerus is convex, but at the elbow, hip and knee the interzones are flat, and it would be difficult at this stage to say, for example, whether the upper ends of the femur and tibia were destined to carry convex or concave surfaces. But at 14 mm. the ends of the cartilages have assumed their characteristic shapes and the nature of the articular surfaces of the larger joints has become clear. The ulna, for instance (Fig. 11, cp. Fig. 8), has newly acquired olecranon and coronoid processes, and a curved interzone separates the convex trochlear surface of the humerus from the sigmoid notch of the ulna. The specimen corresponds closely to Lewis's (1902) 14 mm. stage (embryo CLXIV).

THE CARPAL AND TARSAL REGIONS

At 11 mm. (Lewis, 1902; Bardeen, 1905; embryo CIX) and 12 mm. (Fig. 6), the carpal and tarsal regions are represented by masses of condensed blastema. At the wrist this is continuous proximally with the radius and ulna, and distally with the metacarpals, and in this blastema the elements of the carpus are appearing as centres of more condensed tissue. At 13 mm. (Figs. 9, 10) the carpus and tarsus have diverged in structure from the other parts of the

skeleton, for each element is represented not by a chondrified centre in the interior of the continuous blastema, but by a separate condensation of the blastema itself, separated from the neighbouring elements by intervening sheets of rarefied tissue. By 14 mm. (Fig. 12) the carpal and tarsal elements are chondrified and are separated by dense interzones comparable in structure with those of the larger joints, and thereafter the course of development follows that of other regions.

The appearance of separate centres of condensation for the small bones of the carpus and tarsus is, of course, well known, and has often been figured, particularly in connexion with the study of the homologies of these elements, but the peculiar contrasts in the course of development of these and the long bones do not seem to have attracted attention. Probably they are of no morphological significance, but depend on the relatively late appearance of the cartilage in the carpal and tarsal elements.

CAPSULES AND SYNOVIAL MESENCHYME, 16–19 mm.

At 16 mm. the interzones of the larger joints (Figs. 13, 14, 16) are still homogeneous and continuous with the perichondrium surrounding the cartilages. But on both flexor and extensor surfaces of the humero-radial joint curving strata of condensed tissue are seen passing between the articulating elements, bridging over the joint regions. There can be little doubt that these bulging sheets represent the fibrous capsules and ligaments, though it is surprising to find them so early, for they have not been described in the human embryo at stages before 20 mm. (Bardeen, 1905, embryo XXII). But this 16 mm. embryo is particularly well preserved, and the identification is supported by Hagen-Torn's (1882) description of the capsules in rabbit embryos of 18–20 mm., and Carey's (1922) figure of a pig of 19 mm.

The cells of the capsules are arranged longitudinally, while those of the perichondria near the joints have, as yet, no particular orientation, though towards the shafts of the cartilages the perichondria are already becoming differentiated in preparation for ossification. It will be convenient to speak, in describing this and later stages, of two parts of the perichondrium, the intra- and extra-capsular. Each interzone can then be said to be continuous at its periphery with the intra-capsular perichondrium, and this in turn to be continuous with the extra-capsular perichondrium.

Between the capsule and the extra-capsular perichondrium is a zone of less condensed tissue, as yet poorly developed, the synovial mesenchyme (of Luschka, 1858; vascular 'mesochondrium' of Hagen-Torn, 1882). This tissue will be concerned later with the formation of the synovial apparatus, including synovial and sub-synovial tissues, and in it intra-capsular structures such as ligaments will develop.

Anterior to the knee the quadriceps tendon is already developed at 18 mm. (Fig. 10), and since this takes the place of a fibrous capsule, no separate capsule is developed here (Fig. 16). At the shoulder (Fig. 13) and hip the

capsule cannot yet be distinguished from the dense tissues which will give rise to the fibro-cartilaginous labra and the tendons surrounding the joints.

In the early stages of skeletal development the vascular arrangements are simple, for the blastema itself is completely avascular, a point mentioned by Hagen-Torn (1882) and Retterer (1902), and illustrated in a 10 mm. rabbit embryo by Floderus (1915) in support of his contention that the blastema was of ectodermal origin. In the human 10 mm. embryo (Fig. 1) vessels are seen passing between parts of the blastema which will later form the radius and ulna, but none ramify in the blastemal tissues themselves. Nor do they invade the early derivatives of the blastema, for the early cartilages, perichondria and interzones are quite free of blood vessels (cp. Fig. 11), which come to form characteristic networks over the outer surfaces of the perichondria. Here they are often difficult to distinguish, but in the well-fixed 16 mm. material they can be made out even in the monochromatic reproduction of Fig. 13 as they cover the inner surface of the scapula, and they have been figured covering the tibia of the 20 mm. pig by Carey (1922). No vessel enters the perichondrium itself until this layer alters in structure in preparation for the formation of periosteal bone. But small vessels do pierce the fibrous capsules of the joints and are seen in Figs. 14 and 16, lying in the synovial mesenchyme on the inner side of the capsules, so that, as Hagen-Torn (1882) has pointed out, at this stage the synovial mesenchyme (figured in a 32 mm. sheep embryo, his Fig. 1) resembles the subcutaneous tissue in structure.

At 19 mm. the pattern of the interzone, synovial mesenchyme, capsule and perichondrium is more distinct (Figs. 17, 18, 19), and resembles that shown in Schulin's (1879, Fig. 1) classical figure of an interphalangeal joint at 27 mm. This pattern is probably to be found at an appropriate stage in each joint, but it has seldom been figured (Hesser, 1926, Figs. 2*a*, 3, shows it in the shoulder at 22 mm., and Whillis, 1940, Fig. 1, in a toe at 30 mm.), and its significance has not been discussed. Where strong ligaments will later be associated with the capsule the structure of the embryonic tissue is peculiarly dense (Fig. 19) but the ligaments and capsules cannot yet be distinguished from each other. All the interzones are still perfectly homogeneous dense structures composed of tightly packed rounded cells, as in Lewis's (1902) 18 and 20 mm. embryos (XVII and XXII), and the cells are undergoing frequent mitotic division (Retterer, 1902, Fig. XV, 17 mm. guinea-pig). On their surfaces the interzones are chondrifying, so that at this stage they form important growth zones for the skeleton.

THE THREE-LAYERED INTERZONE AND INTRA-CAPSULAR DIFFERENTIATION, 21–30 mm.

At 21 mm. the interzones of some of the joints have lost their homogeneous character. At the elbow (Fig. 21) both humero-radial and humero-ulnar joints are distinctly three layered, with two dense chondrogeneous layers ('chondrogenen Schichten' of Bernays, 1878), destined to form the articular surfaces of the joint, separated by an intermediate loose layer. This intermediate layer

of the interzone is continuous peripherally with the synovial mesenchyme, and the two are similar in structure, but the interzonal tissue is, and always remains, avascular. Its histological structure has been studied in detail and illustrated in a 30 mm. sheep embryo by Retterer (1902).

The chondrogenous layers are continuous peripherally with the intra-capsular perichondrium, and form with it a complete investment for the end of each skeletal element. These layers persist to a much later stage of development, and at this later stage are often spoken of as true perichondria, which are said to cover the articular surfaces in the embryo (e.g. Bardeen & Lewis, 1901, 30 mm. embryo CCXXII). There is no fundamental objection to such a usage at this stage of development for there is no histological distinction between the chondrogenous layers and the intra-capsular perichondrium, and no sharp topographical distinction can be made once the interzone has become three layered. But since at an earlier stage the interzone seems to be a separate organ, a conception supported by the experimental work of Fell & Canti (1934), it is convenient that its derivatives should be distinguished from neighbouring structures throughout development. Further, the statement that in the embryo the articular surfaces are covered by sheets of 'perichondrium' seems to have misled some workers into the belief that typical perichondria of adult type, provided with abundant coarse collagenous fibres and blood vessels, were to be looked for in embryonic joints. Such a mistake could hardly have arisen if Bernays's conception of chondrogenous layers of the interzone had become established.

The shoulder joint at 21 mm. shows an early stage of the loosening of the middle layer of the interzone (Fig. 20), reported by Simon (1923) at 18 mm., though the interzone is still homogeneous in Hesser's 22 mm. embryo (1926) both at the shoulder (his Fig. 3) and at the hip (his Fig. 18). The capsule of the shoulder and the tendons nearby are now differentiated from the dense mesenchyme seen at an earlier stage, but the synovial mesenchyme is scanty. At the knee (Fig. 22, and Hesser, 1926, Fig. 19, 22 mm.), dense interzonal tissue still intervenes between each femoral condyle and the corresponding articular surface of the tibia. By 23 mm. the interzone of the shoulder (Fig. 23) has attained the typical three-layered structure and the fibro-cartilaginous labrum is now conspicuous. This latter is continuous centrally with the chondrogenous layer of the interzone which covers the glenoid surface of the scapula and peripherally with the perichondrium of the neck of the scapula, and would appear to be developed from the interzonal tissue. The continuity with the interzone is very clear in a 24 mm. embryo (Fig. 24), in which the staining is unusually sharp. At the hip and knee the interzones are still dense and homogeneous.

At 26 mm. the interzones of the knee are in the early three-layered stage (Fig. 25, and Langer, 1929, 26 mm. embryo), and the menisci are indicated as faint independent condensations in the synovial mesenchyme.

At 29 mm., just before the joint cavities appear, the distinction of the layers

of the interzone becomes sharper (Fig. 29). In the intermediate layer the cells become flattened and lie with their surfaces parallel to the surfaces of the interzone, and at the same time the matrix of this tissue becomes clearer in preparation for liquefaction. At the shoulder (Fig. 28), whose development at this stage is a little in advance of that of the other joints, the synovial mesenchyme on either side of the joint is actually breaking down and small cavities are already formed. Towards the centre of this joint the interzonal tissues present a peculiar appearance, for the intermediate layer of the interzone is represented not by a loose tissue, but by a dense layer in which the flattened cells are close-packed and often aggregated into clumps. If these cells were absent it would seem as if the interzone had reverted from a three-layered to a homogeneous structure, yet it is improbable that such very different structures as those shown in Figs. 28 and 29 should co-exist in the same preparation. It is more likely that the appearances at the shoulder joint are artifacts due to the compression of the soft intermediate layer of the interzone by the expansion of the cartilages during the preparation of the material, and a pressure fold is actually seen crossing the neck of the scapula near the joint. A similar contrast in structure has been figured without comment in the humero-ulnar and humero-radial joints at 27 mm. by Hesser (1926, Figs. 7*a*, *b*) and here also the pressure folds are well developed in the sections figured.

By 29 mm. all the larger joints have reached the stage in which the interzone is three layered. At the shoulder this was attained at 23 mm. (Fig. 23) and at the elbow at 21 mm. (Fig. 21), and Hesser (1926) found it at the elbow at 25 mm. (his Fig. 6) and 27 mm. (his Fig. 7). The interzones of the wrist and most of the intercarpal joints are three layered at 25 mm. (Hesser, 1926, Fig. 10), and all the interzones of the fingers by 27 mm. (Hesser, 1926; cp. my Fig. 20 and Floderus, 1915, Fig. 35). Those of the toes are still homogeneous at 30 mm. (Floderus, 1915, Fig. 22; Whillis, 1940, Fig. 1), but three layered at 32 mm. (Hesser, 1926, Fig. 32). The hip is three layered at 30 mm. (Hesser, 1926, Fig. 32; Bardeen, 1905, embryo CCXXVII), the knee at 26 mm. (Langer, 1929), the ankle and talocalcanean joints at 27 mm. (Hesser, 1926, Figs. 26, 30), and the smaller intertarsal joints, still homogeneous at 27 mm., are three layered at 32 mm. (Hesser's Fig. 27; cp. my Fig. 42). The interzone between the temporary cartilage in the fibrocartilage of the wrist (possibly a primary element of the carpus, Thilenius, 1896 and Corner, 1898), and the styloid process of the ulna never passes beyond the homogeneous stage, which is still retained at 49 mm. (Fig. 48). Hesser has described a three-layered stage in the intervertebral and costo-vertebral joints at 25 mm., and it is reasonable to suppose that all synovial joints pass through this stage.

Yet, though Bernays (1878) described the three-layered structure in detail, he failed to illustrate it, and it has often passed unnoticed by later authors. Whillis (1940) ignored it altogether, and indeed his Fig. 6, from a wrist at 30 mm., shows interzones which appear to be homogeneous. But since Hesser's

(1926) 25 mm. and my 30 mm. (Fig. 34) and 34 mm. embryos show the three-layered structure clearly there can be little doubt that the appearances illustrated by Whillis are due to artifacts and are of similar nature to those illustrated in my Fig. 28. In the interphalangeal joints, too, Whillis did not find the three-layered stage at the time when it was presumably present (his Fig. 2), though it certainly does occur in these joints (Hesser, 1926, Fig. 32, and my Fig. 37).

The intra-capsular structures are, so far as is known, all formed from condensations of the synovial mesenchyme (Bernays, 1878; Hagen-Torn, 1882). At the shoulder the long head of the biceps can be distinguished as a condensation within the fibrous capsule at 20 mm. (Simon, 1923) and 22 mm. (Neale, 1937), and the course of embryological development gives no suggestion of any migration of the tendon through the capsule, such as is believed, from anatomical evidence, to have occurred in the course of phylogeny. Similarly, the ligamentum teres of the hip is believed to have migrated from its reptilian position outside the joint, where it functioned as a collateral ligament, to its mammalian position inside the joint cavity (Moser, 1893), yet in the rabbit it arises *in situ* as a condensation which can be recognized at 15 mm. (Schuster, 1880) and 18 mm. (Hagen-Torn, 1882), while in the human embryo it is more difficult to distinguish from other condensed tissues at early stages, but it has been figured in a vascular synovial mesenchyme at 22, 30 and 34 mm. (Schuster, 1880; Moser, 1893; Bardeen, 1905; cp. my Fig. 38).

At the knee the intercondylar interval is filled with an abundant vascular synovial mesenchyme, and in this tissue the cruciate ligaments appear as poorly marked condensations, first visible at 20 mm. (Bardeen, 1905, embryo CCXXIX; Walmsley, 1940, Fig. 1; cp. my Fig. 26). The menisci appear as independent condensations separate from the femur, tibia and capsules (Langer, 1929, and my Figs. 25, 39), not as secondary derivatives of the capsule, as suggested by Luschka (1855). The intra-capsular part of the tendon of the popliteus, studied in great detail by Moser (1892, unillustrated), appears in its definitive position in a loose mesenchymal tissue (Fig. 41), and the general cavity of the knee joint gradually extends both above and below the lateral meniscus so as to surround the tendon in later development. The fibrocartilaginous disc of the wrist joint (Whillis, 1940, Fig. 6, and my Fig. 48), and the interosseous ligaments of the carpus (Fig. 49) are, at the time of their formation, surrounded by an abundant loose vascular mesenchymal tissue, though later they are intimately related to the joint cavities.

THE JOINT CAVITIES AND SYNOVIAL TISSUES AT 30 mm. AND ONWARDS

At 30 mm. (Figs. 31, 32) the middle layer of the interzone and the inner portions of the synovial mesenchyme are softening, and in part already broken down to give the first joint cavities. The process of liquefaction was studied carefully by Retterer (1896) in material specially fixed for the purpose

from the bursae mucosae, where the preparations were not complicated by the presence of cartilage or bone, and later (1902) he confirmed his conclusions by the study of typical synovial joints. He found that the intercellular ground substance was liquefied so as to leave a cellular network composed of scattered strands containing irregularly disposed nuclei. Some of the cells were eventually destroyed, but most became attached to one or other of the walls of the cavity, and persisted as its lining. This account is confirmed by special preparations of well-fixed material made by Dr Hughes (Fig. 50), in which the synovial surface is ragged, and strands of tissue float out from the surface into the synovial cavity. Some of the cells in the interior of the cavity appear to be dying, and the synovial fluid contains cellular debris.

As the cavities develop the sections become more difficult to interpret. The humero-radial (Fig. 32) and humero-ulnar (Fig. 33) joints are taken from neighbouring sections of the same serial preparation, yet they look very different. Between the humerus and radius is loose liquefying tissue, and each articular surface is clothed by its own chondrogenous layer, while the humerus and ulna appear on the contrary to be joined by a homogeneous interzone in which the cells are compressed into a single dense layer. But since at both earlier (Fig. 29) and later (Fig. 36) stages a three-layered structure of the interzonal tissues may be observed at the humero-ulnar joint, there is little doubt that the type of appearance shown in Fig. 33 is the result of an artifact caused by pressure between the articulating elements. Cartilages are notorious for their liability to expand and contract as they pass from one solution to another both in the cellodion and paraffin techniques, and pressure folds and even overlapping of the articular surfaces are all too common in the sections. At this stage it is relatively easy, however, to decide what appearances are natural and what due to artifact, but in later stages the difficulties are enormously increased.

At 34 mm. the cavity of the humero-radial joint is well formed (Fig. 35) but still contains a few cells in its interior, and a separate joint cavity is forming between the head of the radius and the annular ligament, but the humero-ulnar joint (Fig. 36) is less advanced, with occasional isolated areas of liquefaction in the loosened tissue. At the hip (Fig. 38) the cavity is spreading round the head of the femur and the ligamentum teres lies in the synovial mesenchyme accompanied by conspicuous blood vessels which will later supply the cartilage canals of a large part of the head of the femur (Haines, 1933). At the knee the menisci are now sharply differentiated and joint cavities have developed between the anterior parts of the menisci and the femoral condyles (Fig. 39 and Floderus, 1915, Fig. 42). Reconstructions showing the precise distributions of these cavities have been made by Langer (1929).

The joint between the patella and femur calls for special comment. At 13 mm. (Fig. 10) the quadriceps passes to the tibia as a band of dense tissue quite distinct from the femur, and separated from it by a layer of loose

uncondensed tissue. At 20 mm. (Walmsley, 1940, Fig. 1) the patella is differentiating as an aggregation of round cells in the quadriceps mass, and at 23 mm. it is more distinct (his Figs. 2, 3), but the arrangement of the quadriceps tendon and its relation to the femur are unchanged. At 32 mm. (Walmsley, Fig. 5; Hesser, 1926, Fig. 22*b*) the patella is separated from the femur by very loose tissue but there are as yet no joint cavities separating them, and in a sheep embryo of 52 mm. total length, though the patella was not yet ossified, Kazzander (1894) found a similar tissue (his 'stratum intermedium') between the femur and quadriceps tendon. So far there is no difficulty in the accounts, though the femoro-patellar joint differs from the more typical joints in that no dense interzone was found at any stage, so that the loose tissue which separates the parts at 32 mm. has persisted as such from an early stage of development. But now both Kazzander and Walmsley describe a disappearance of the loose tissue between the patella and femur so that their perichondria become fused, a condition shown in Kazzander's sheep embryo of 57 mm., and Walmsley's human embryos of 35 and 38 mm., and this is interpreted by Walmsley as indicating a late secondary formation of an interzone (his 'articular disc'), similar to those of other joints, and he suggests that this structure plays an important part in the definition of the articular surfaces. Yet in my 34 mm. preparation (Fig. 40) there is no fusion whatever between the patella and femur; instead there is a broad layer of loose tissue in which a small but typical joint cavity has appeared, and Hesser's (1926, Fig. 25*a*) 40 mm. specimen has a similar structure but the joint cavity is larger. Walmsley's Fig. 4 would appear to have been taken from poorly fixed material, and the frequency with which artifacts have been seen to appear in developing joints wherever the dense articulating elements are separated by soft liquefying tissue makes it probable that Kazzander and Walmsley have been misled by similar artifacts due to compression of the loose tissues between the patella and femur.

Judging from my series the cavities of the larger joints may be said to appear at or soon after the onset of periostial ossification in the long bones, an estimate supported by other workers. Black (1934) found a small split of the shoulder at 32·5 mm., Kessel (1927) found the iliopsoas bursa present but the cavity of the hip joint absent at 28 mm., and Moser (1893) found a cavity absent at 30 mm. but present at 34 mm. At the knee Langer (1929) found no cavities at 28 mm., Bardeen (1905) and Floderus (1915) early cavities at 30 mm., and Langer several cavities at 37 mm. In the smaller joints the cavities appear later. At 34 mm. the ankle and intertarsal joints still have typical three-layered interzones though the synovial tendon sheaths have already appeared (Fig. 42), and Hesser (1926) found a similar structure at 40 mm.

DEHISCENCE OF THE ARTICULAR SURFACES

There is no particular difficulty in the determination of the time of the first appearance of the joint cavities in the synovial mesenchyme but as regards

the time of final separation of the cartilages there are very wide discrepancies between the observations of various authors, and no precise estimates can be abstracted from the literature. This state of affairs is due to the difficulties of interpretation of preparations from joints, and these must now be considered in some detail.

The humero-radial joint has been followed through stages with a homogeneous interzone (Figs. 4, 14) and a three-layered interzone (Fig. 21), through the stage of early liquefaction (Fig. 32) to the stage of full separation at 34 mm. (Fig. 35), when the cavity is clear but for a few scattered strands of cells, and other preparations examined in the course of this investigation agree well with those illustrated. But at 45 mm. (Fig. 43), though the joint cavities are perfectly distinct peripherally, the more central parts of the articular surfaces of the humerus and radius appear to be joined by cartilage. The general preservation of the specimen is good, and there is nothing to suggest at first sight the presence of a major artifact. Yet a high-powered view (Fig. 44) shows that near the region of contact each articular surface is covered by a thin, deeply staining, fibrillar layer, similar to that illustrated in Hammar's (1894) classical figure of articular cartilage from a newly born subject, and lying in and below the fibrillar layer are flattened cells, contrasting with the more rounded cells deeper in the cartilage. Where the cartilages meet, the fibrillar layers fuse to form a single sheet which can be found throughout the area of apparent fusion, and on either side of this sheet are flattened cells. Some of these cells appear to be degenerating, and small masses, probably debris derived from such cells, are found in and near the fibrillar layer. The curvatures of the articular surfaces are irregular, for the surfaces suddenly bend towards each other just before they meet and then pass into a smooth curve over the region of contiguity.

Comparing this arrangement with that seen earlier it would appear that the chondrogenous layers of the interzone, already inconspicuous at 34 mm., have now become completely chondrified, so that their identity is lost, and that the cuticular layers and flattened cells are the remnant of the intermediate loose layer. The changes of curvature in the articular surfaces suggest that during the preparation of the sections the cartilages, originally separate, swelled so as to press against each other, and in the more central regions of the joints became stuck together, and that later the cartilages shrank away from each other except where they were attached. On the other hand the peculiar shapes might be due to a primary continuity partially broken by tearing due to shrinkage.

Hultkrantz (1897) referred to the difficulties met with in the interpretation of joint sections. He said that *a priori* it would seem that there should be no difficulty in determining the presence or absence of a joint cavity, but when in a single serial preparation the cavity appeared open in one section and closed in a neighbouring section it was difficult to decide whether there had been a secondary fusion of the cartilages or a tearing apart of originally continuous

structures. This kind of variation is illustrated in the 45 mm. embryo. In the third metacarpo-phalangeal joint, for instance (Fig. 46), the cartilages are apparently united, in the fifth (Fig. 47) from the same series of sections they are separate, yet it is most unlikely that the fifth digit would really be in advance of the third in its development. Material from more advanced embryos presents similar difficulties.

Other authors have accepted the apparent absence of a cavity at its face value. Schulin (1879) described the cartilages of the elbow at 70 mm. and Floderus (1915) those of a metacarpal-phalangeal joint at 50 mm. as still united, and Whillis (1940) speaks of complete cartilaginous union at later stages. There can, however, be no doubt that articular surfaces can become adherent in sections prepared with ordinary care. Dr Richardson's stock series at University College from the joint of a kitten that had been running about for some days, though very well fixed and prepared, shows numerous local adhesions, and my own preparations from adult material of agile South African lizards show similar appearances, which must be artifacts. A dissection of the joints of the 45 mm. embryo with the aid of a dissecting microscope showed the cartilages of all the large joints completely free from each other, and this was confirmed by examination of older embryos. Though some doubt may remain, it is consistent with the evidence to believe that the intermediate layer of the interzone is always liquefied soon after the synovial mesenchyme, so that dehiscence is complete by the 34 mm. stage in the shoulder and humero-radial joints (Figs. 31, 35), by 44 mm. at the knee (Hesser, 1926, Fig. 25*a*; cp. my Fig. 45) and probably at the hip (open in my 45 mm. embryo), by 45 mm. at the metacarpo-phalangeal joints (Fig. 47) and ankle joints (my 45 mm. embryo, not yet in Hesser's 40 mm. embryo), while the wrist joints and carpal joints do not dehisce until after 50 mm. (Fig. 49).

COMPLETION OF THE SYNOVIAL CAVITY

When the joint cavities are first formed they extend only a short distance into the synovial mesenchyme, and have none of the complex recesses they will develop later. In the humero-radial joint, for instance (Figs. 32, 35), the cavity follows the articular surface of the capitulum of the humerus, but does not yet include the head of the radius. The cavity between the annular ligament and head of the radius is formed separately, and is seen in the course of liquefaction at 34 mm. (Fig. 35) and fully developed and continuous with the main synovial cavity of the extensor surface of the joint at 45 mm. (Fig. 43). With the extension of the cavity over the humerus and radius, parts of the synovial mesenchyme are demarcated as synovial folds (Fig. 43; cp. Lucien, 1904, folds of the knee), which become progressively more conspicuous with the spread of the cavities (Fig. 54; cp. Henke & Reyher, 1874, Fig. 25, metacarpo-phalangeal joint), and are prominent features at birth (Grant, 1931; Santo, 1937).

At the knee the early cavities follow the condyles of the femur (Hesser, 1926, 40 mm. embryo and my Fig. 45), not the tibia, so that at this stage the menisci

are separate from the femur, but joined to the tibia by loose synovial mesenchyme, liquefying in my 45 mm. embryo, and destroyed in Floderus's (1915) 50 mm. specimen. Though the menisci were recognizable as distinct condensations of the synovial mesenchyme before the synovial cavities appeared (Fig. 25), their final separation from the articular cartilages resembles closely that of the synovial folds of the humero-radial joint, an observation which supports Grant's (1931) conclusion that the two structures are morphologically comparable with each other. The femoro-patellar cavity, first seen at 34 mm. (Fig. 40), was found continuous with the condylar cavities at 47 mm. by Langer (1929), and he and Moser (1892) have discussed the later expansion of these cavities and the inclusion of the 'supra patellar' bursa as a part of the knee joint.

THE ARTICULAR SURFACES

Hesser (1926) has collected together the various theories that have ascribed the shapes of the articular surfaces to the direct or indirect results of embryonic movements, and has rejected them on the grounds that the shapes are already developed at a stage when the interzones are still homogeneous, and little or no movement could occur. It is, however, possible that the appearance and later extensions of the joint cavity may be associated with embryonic movement, for in the rat embryo angular movements of the elbow joint are seen at 13 mm., at a stage when the interzone is three layered (Blincoe, 1928). But the course of development of the malleolo-incudal joint, where movements must be small or absent before birth, appears quite similar to that of the other joints of the body (Urbantschitsch, 1880).

At the time of their first appearance the synovial cavities are formed partly in the tissues of the interzone and partly in the synovial mesenchyme (Figs. 32, 35), so that the articular surfaces are composed centrally of the chondrogenous layers of the interzones, and peripherally of tissues of similar constitution which were originally part of the intra-capsular perichondrium. With the further spread of the synovial cavities (Figs. 43, 54), a large proportion of the perichondrium becomes chondrified so as to form articular cartilages, and the greater parts of the articular surfaces of most joints are formed in this way. Near the margins of the articular surfaces there is a zone of transition between the articular surfaces and perichondrium (Fig. 56), which progresses over the embryonic cartilages so long as the articular surface is spreading, but eventually becomes stationary to form the transition zone of the adult, described in detail by Key (1932).

The histological structure of the articular surfaces varies at different ages and in different joints. At the stage of liquefaction (Fig. 32) the tissues forming the surfaces are not yet chondrified, so that the chondrogenous layers of the interzones are still recognizable as such, and similarly, where the cavities are forming in the synovial mesenchyme the articular surfaces are still covered by perichondrium, as is the head of the radius where it articulates with the annular

ligament (Fig. 43). With full chondrification the remains of the liquefied tissues of the interzone or synovial mesenchyme come to form, as we have seen, a thin fibrillar layer overlying the cartilage, containing flattened cells some of them pyknotic or reduced to debris (Fig. 44). Eventually these flattened cells disappear, so that now if the articular surfaces have come into contact in the course of preparation of the material there is no striking change in cell type as the structure of the cartilage is followed from one articulating element to another. This is the stage figured by Whillis (1940) in an interphalangeal joint at 125 mm. and in a newborn rat to illustrate union of the articular cartilages by 'primitive cartilage'. At earlier stages, however, artifacts of this nature have been found to occur with some frequency, and the appearances in Whillis's preparations (similar to those illustrated in my Figs. 58, 59) were probably also due to such artifacts. Floderus (1915, Figs. 29, 30) has illustrated complete joint cavities in the rat embryo at 18 mm., and the recognition that apparent union of the cartilages may be due to artifact solves the paradox that embryonic movements are well developed at a time when the cartilages may be apparently joined.

The fibrillar layers persist till birth (cp. Fig. 55), at which time the main mass of the cartilage is still homogeneous in appearance. The development of the various layers of the adult articular cartilages (sliding, transitional, pressure and basal), described by Benninghoff (1925) and others, must occur later and has not yet been studied.

In some joints the articular surface is formed not of hyaline but of fibro-cartilage, and this distinction is quite evident in the embryo, e.g. the glenoid surface of the scapula is covered with a layer of fibro-cartilage at 66 mm. (Fig. 51). The acromio-clavicular joint at this stage is largely filled by a mass of dense fibro-cartilaginous tissue, still attached to the clavicle, but separated from the acromion by a fully developed joint cavity. Later much of this tissue will be separated from the clavicle to form the inter-articular disc, but some will be left to form the fibro-cartilaginous sheet covering the clavicle itself. The jaw joint is similar in its development, for here at 47 mm. and 65 mm. Hesser (1926) found a joint space below the disc, but none between the disc and the temporal bone, and here again the articular surfaces are covered with fibro-cartilage (Luschka, 1858).

SYNOVIAL AND EXTRA-SYNOVIAL STRUCTURES

The structure of the synovial surface of any particular region depends, as was pointed out by Langer (1929) and others, on the rate of spread of the synovial cavity, as well as on the nature of the substratum on which the surface lies. At the time of the first appearance of the joint cavity the synovial surfaces are ragged (Fig. 32), but as soon as active extension of the cavity slows down, even temporarily, the surface is smoothed off and is covered with a layer of synovial cells (Figs. 35, 43, 45). By the 60–70 mm. stage most of the joints have attained the proportions they will have in the adult (Fig. 51), and the

synovial surfaces are formed of one or two layers of typical synovial cells lying on the vascular substratum (Fig. 52). In the smaller joints and in the recesses of the larger joints, however, there is still evidence of extension in this and later stages, for the dorsal recesses of the inter-phalangeal joints at 125 mm. (Fig. 53), and the recess of the humero-radial joint which projects distally to the annular ligament at 66 mm., still show the typical ragged surfaces which indicate advancing liquefaction.

Where the synovial surface overlies dense structures such as ligaments and tendons without the intervention of loose connective tissue, the synovial tissues are poorly developed. The inner surface of the annular ligament, for instance, is separated from the head of the radius by a layer of loose synovial mesenchyme at 30 mm. (Fig. 32), and when this liquefies its remnant forms a single layer of synovial cells covering the inner surface of the ligament at 34 mm. (Fig. 35) and 45 mm. (Fig. 43), and similar cells can be seen covering the upper surface of the menisci at the knee (Fig. 45), and the inner surfaces of the volar accessory and collateral ligaments of the digital joints (Figs. 46, 47). This synovial stratum can still be distinguished at later stages (Fig. 56), but becomes progressively less differentiated, and in the adult can hardly be distinguished (Petersen, 1930; Key, 1932).

In the earlier stages the synovial cells lie directly on the loose synovial mesenchyme, but later there may be some condensation of the connective tissue to form a distinct tissue, the stratum sub-synovial (Fig. 56). In most joints this is indistinct, but at the knee it is eventually well developed in the region of the intercondylar septum, and can be dissected away as a continuous sheet. It is, of course, formed much later than the fibrous capsule for it is barely visible at 45 mm., whereas the fibrous capsule appears at 16 mm. (Fig. 16), and it does not in any way deserve the name of 'true' capsule, given it by Higgins (1896).

The fibrous capsules and ligaments all appear as mesenchymal condensations (Fig. 14, capsule of humero-radial joint; Fig. 19, collateral ligaments of digital joints). Where tendons or ligaments are developed in close association with the capsule they are not at first distinguishable from it, but later their histological structure becomes differentiated. In a humero-radial joint from a specially well fixed rat embryo of 20 days (Fig. 57), the radial collateral ligament, passing, as it does in this animal, between the humerus and radius, already shows the characteristic spiral twisting of its fibres, and its cells are cuboidal in shape and arranged in longitudinal rows between the straight fibre bundles of the ligament. The same kind of structure is seen in the tendons of origin of the muscles arising from the humerus, but the fibrous capsule of the joint has quite a different structure with small flattened cells, either scattered or arranged in short rows between the wavy fibre bundles. The sharp contrast in gross structure between ligaments and fibrous capsules has been described in the adult (Haines, 1944); this specimen shows that the contrast is recognizable in the microscopic structure and is developed in embryonic life.

At the time of liquefaction the interzone is of about the same thickness throughout its extent, so that the articular surfaces, when they are first defined, are necessarily congruent (Figs. 32, 33). Later the surfaces develop their characteristic incongruencies so that highly curved convexities always articulate with less curved concavities (Figs. 51, 53, 54), and the area of contact is relatively reduced, probably to satisfy the demands of lubrication (MacConaill, 1932). The gaps between the articular surfaces are filled partly by the synovial fluid and partly by the folds carved out from the synovial mesenchyme by the expansion of the synovial cavities. Later, in most joints, synovial villi are found (Figs. 54, 55). Retterer (1902) considered that these villi were developed directly from the cells found on the rough synovial surfaces at the stage of liquefaction, but since at an intermediate stage the synovial surface is smoothed off this cannot be so, and they are presumably new ingrowths from the synovial tissues. Bennett, Waine & Bauer (1942) have shown that the villi continue to grow throughout life and Meyer (1922) that they appear in chronically inflamed synovial bursae, but very little is known of their development or significance.

THE EMBRYOLOGICAL APPROACH TO THE MORPHOLOGY OF JOINTS

Baer (1837), working on the chick, made the fundamental observation that in general each element of the appendicular skeleton was originally laid down as a separate cartilage, and that the unchondrified tissue between the elements formed the joints. Bruch (1851), who gave the first detailed account of the development of the joints themselves, described the formation of the synovial tissue and joint cavities and suggested that the fibrous capsule was a continuation of the perichondrium across the joint region. Schulin (1879) confirmed this suggestion. His Fig. 1, a section from an inter-phalangeal joint of a 26 mm. foetus, shows a structure similar to my Fig. 19, with a homogeneous interzone, a fibrous capsule enclosing the synovial mesenchyme and intra- and extra-capsular perichondrium. All these structures he derived from the blastema, so that he considered the synovial mesenchyme as blastemal tissue loosened in preparation for the formation of the joint cavity. Lewis (1902) and Bardeen (1905) accepted Schulin's suggestions without question and they are found in all modern text-books concerned with joint morphology.

Yet there are considerable difficulties in the way of accepting Schulin's ideas. If the fibrous capsule really represented the perichondrium it would be surprising to find another layer, the intra-capsular perichondrium, enclosed within it. The perichondrium near the larger joints is perfectly clear at 12 mm. (Fig. 4), while the fibrous capsule is still absent at 14 mm., and when it does appear, by 16 mm. (Fig. 16), its cells are arranged longitudinally while those of the perichondrium are rounded. Further, Hagen-Torn's (1882) discovery that the synovial mesenchyme was vascular, a point confirmed by my Figs. 14 and 16, led him to assert that it was a part of the general mesenchyme, cut

off by the development of the joint capsule, and not a part of the avascular blastema, so that he saw in the fibrous capsule a completely extra-blastemal formation. Unfortunately his work was poorly illustrated and has never been discussed. My own work supports Hagen-Torn's theory rather than Schulin's, for where the development of the joints is uncomplicated by neighbouring structures it is clear that the capsules are new formations. The tissue filling the bend of the elbow and knee at 12 and 13 mm. is loose (Figs. 4, 10), and in it the capsules appear at 16 mm. (Figs. 14, 16). At 16 mm. the metacarpophalangeal joints are surrounded by loose tissue which fills the indentations between the two cartilages (Fig. 15), and the capsules appear in this tissue (Fig. 19). It is difficult to believe that in either of these cases the synovial mesenchyme can be blastemal in origin though in the case of the shoulder (Fig. 20), when the synovial mesenchyme is less abundant, its origin cannot be determined with certainty.

It is possible that the simpler joints without synovial cavities or synovial apparatus, such as are found commonly in fishes (Haines, 1942) and have survived in the joints between the body and cornua of the mammalian hyoid, are blastemal in origin, but the more complex synovial joints, first developed in the jaw joints of gnathostomes, include derivatives from the surrounding general mesenchyme.

SUMMARY

1. The development of the joints of the limbs is described from the time of their first definition at 11 mm. to the time of the appearance of the synovial cavities of the larger joints at 30–34 mm., and some stages from later embryos are discussed.

2. The joints first appear as interzones, which are formed from the remains of the skeletal blastema between the cartilages. The interzones form the more central parts of the articular cartilages and synovial cavities.

3. Each interzone passes through a three-layered stage with two chondrogenous layers and an intermediate loose layer, and a stage where the intermediate layer breaks down and the chondrogenous layers become fully chondrified.

4. The development of the fibrous capsules as condensations in the extra blastemal tissue near the joints cuts off a part of the general mesenchyme to form the synovial mesenchyme, and a part of the perichondrium to form the intra-capsular perichondrium.

5. The synovial mesenchyme gives rise to the more central parts of the synovial cavities, the synovial and sub-synovial tissues and all intra-capsular structures including ligaments, tendons and fibro-cartilages.

6. The intra-capsular perichondrium is partly transformed into the more peripheral parts of the articular cartilage, while the remainder persists throughout life.

7. The interzones of the larger joints appear at 11–12 mm., their fibrous capsules at about 16 mm., the interzones become three layered at 21–26 mm., liquefaction at the synovial mesenchyme begins at 30–34 mm., and dehiscence of the articular cartilages is complete by the 40 mm. stage but is delayed at the wrist and ankle.

8. When the synovial cavities are first formed the remnants of the liquefying tissue at first cover the articular surfaces of the cartilages, but soon are reduced to form narrow cuticular layers containing a few flattened cells, and before birth all the flattened cells disappear.

9. At the stage of liquefaction, and wherever the joint cavities are spreading rapidly, the synovial surface is ragged, and strands of cells project into the synovial cells of the joint, but when extension is arrested, even temporarily, a smooth layer of synovial cells demarcates the cavity.

10. The synovial folds are part of the synovial mesenchyme dissected out by the secondary extensions of the cavities as they spread over the cartilages. The villi are formed as secondary ingrowths of the synovial tissues.

11. A revision of joint development supports the morphological conceptions of Hagen-Torn rather than those of Bruch and Schulin.

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REFERENCES

BAER, K. E. v. (1837). *Ueber Entwickelungsgeschichte der Thiere*, II. Theil. Königsberg: Borntträger.
BARDEEN, C. R. (1905). *Amer. J. Anat.* **4**, 265.
BARDEEN, C. R. & LEWIS, W. H. (1901). *Amer. J. Anat.* **1**, 1.
BENNETT, G. A., WAINE, H. & BAUER, W. (1942). *Changes in the Knee Joint at various Ages.* New York: Commonwealth Fund.
BENNINGHOFF, A. (1925). *Z. Zellforsch.* **2**, 783.
BERNAYS, A. (1878). *Morph. J. b.* **4**, 403.
BLACK, B. M. (1934). *Anat. Rec.* **60**, 333.
BLINCOE, H. (1928). *Anat. Rec.* **40**, 277.
BRUCH, C. (1851). *Denkschr. Schweiz. naturf. Ges.* **12**, 14.
CAREY, E. J. (1922). *J. Morph.* **37**, 1.
CORNER, E. M. (1898). *J. Anat., Lond.*, **32**, 272.
FELL, H. B. & CANTI, R. G. (1934). *Proc. Roy. Soc.* B, **166**, 316.
FLODERUS, B. (1915). *Kgl. Swensk Vetensk. Akad. Handl.* N.F. **53**, 416.
GRANT, J. C. B. (1931). *Brit. J. Surg.* **18**, 636.
HAGEN-TORN, O. (1882). *Arch. mikr.-anat.* **21**, 591.
HAINES, R. W. (1933). *J. Anat., Lond.*, **68**, 45.
HAINES, R. W. (1942). *J. Anat., Lond.*, **77**, 12.
HAINES, R. W. (1944). *J. Anat., Lond.*, **78**, 44.
HAMMAR, J. A. (1894). *Arch. mikr.-anat.* **43**, 266, 813.

HENKE, P. J. W. & REYHER, C. (1874). *S.B. Akad. Wiss. Wien* (Abt. 3), **70**, 217.
HESSER, C. (1926). *Morph. J. b.* **55**, 489.
HIGGINS, H. (1896). *J. Anat., Lond.*, **30**, 289.
HULTKRANTZ, J. W. (1897). *Das Ellbogengelenk und seine Mechanik.* Jena: Fischer.
KAZZANDER, G. (1894). *Arch. Anat. Physiol., Lpz.*, p. 161.
KESSEL, F. (1927). *Morph. Jb.* **58**, 413.
KEY, J. A. (1932). 'The synovial membranes of joints and bursae.' In Cowdray, *Special cytology*, 2nd ed., vol. **2**. New York: Hoeber.
LANGER, M. (1929). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **89**, 83.
LEWIS, W. H. (1902). *Amer. J. Anat.* **1**, 145.
LUCIEN, M. (1904). *Bibliogr. anat.* **13**, 133.
LUSCHKA, H. (1855). *Arch. Anat. Physiol. wiss. Med.* p. 481.
LUSCHKA, H. (1858). *Die Helbegelenke des menschlichen Korpers.* Berlin.
MACCONAILL, M. A. (1932). *J. Anat., Lond.*, **66**, 210.
MEYER, A. W. (1922). *J. Bone Jt Surg.* **4**, 491.
MOSER, E. (1892). *Morph. Arb.* **1**, 147.
MOSER, E. (1893). *Morph. Arb.* **2**, 36.
MURRAY, P. D. F. (1926). *Proc. Linn. Soc. N.S.W.* **51**, 187.
NEALE, R. M. (1937). *Anat. Rec.* **67**, 205.
PETERSEN, H. (1893). *Arch. Anat. Physiol., Lpz.*, p. 67.
PETERSEN, H. (1930). 'Die Organe des Skelettsystems.' In v. Möllendorff, *Handb. mikr.-anat. Mensch.* Berlin: Springer.
RETTERER, E. (1896). *J. Anat., Paris*, **32**, 256.
RETTERER, E. (1902). *J. Anat., Paris*, **38**, 473, 580.
SANTO, E. (1937). *Anat. Anz.* **85**, 223.
SCHULIN, K. (1879). *Arch. Anat. Physiol., Lpz.* (Anat. Abt.), p. 240.
SCHUSTER, H. (1880). *Mitt. embryol. Inst. Wien* **1**, 199.
SIMON, S. (1923). *S.B. Akad. Wiss. Wien* (Abt. 3), **130/31**, 61.
THILENIUS, G. (1896). *Morph. Arb.* **5**, 1.
URBANTSCHITSCH, V. (1880). *Mitt. embryol. Inst. Wien*, **1**, 229.
WALMSLEY, R. (1940). *J. Anat., Lond.*, **74**, 360.
WHILLIS, J. (1940). *J. Anat., Lond.*, **74**, 277.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. 10 mm. Lucas Keene's '2387'. 50.4. Fore-limb. The skeletal blastema (*sk.*) shows clearly in the humeral, elbow (*el.*) and fore-arm regions, while distally it fades away towards the marginal vein. The pre-muscle masses of the upper arm on both flexor (*fl.*) and extensor (*ex.*) aspects, the brachial plexus (*pl.*) and median nerve (*md.*) are visible. A blood vessel of the interosseous group (*b.v.*) pierces the blastema.

Fig. 2. 10·5 mm. Smout's 5½ weeks embryo, 11.1.4. Fore-limb. The pre-muscle masses of the lower arm have now differentiated, and the boundaries of the skeletal blastema are less easy to follow, as the condensations for the skeleton and the muscles now form a continuous mass.

Fig. 3. 10·5 mm. Smout's 5½ weeks embryo, 16.1.4. Hind-limb. The skeletal blastema of the femoral (*fm.*), tibial (*tb.*) and foot-plate (*f.p.*) regions are distinguishable. The pre-muscle masses of the thigh (*p.m.*) are more condensed than the general mesenchyme, but those for the more distal part of the limbs have not yet appeared.

Fig. 4. 12 mm. Smout's 6 weeks embryo, 31.3.5. Humero-radial joint. The lower end of the cartilaginous humerus (*hm.*) is assuming its characteristic shape and the shaft of the radius (*rd.*) is well developed, but the ends are not yet chondrified. A thick interzone (*i.z.*) separates the two chondrifications and gives the first indication of joint formation. Peripherally the interzone is continuous with the dense mesenchyme surrounding the cartilages which now forms their perichondria (*p.c.*). Distally the blastema of the radius is quite continuous with that of the

carpal region and with the condensed tissue which will form the muscular and tendinous apparatus.

Fig. 5. 12 mm. Smout's 6 weeks embryo, 49.2.5. Thigh. The shaft of the femur (*fm.*) is well developed, but that of the tibia (*tb.*) is barely recognizable as a small centre of chondrification. The knee appears as a thick interzone (*i.z.*) but at the hip the femoral tissues are continued without interruption into the dense blastema of the pelvis (*pl.*).

Fig. 6. 12 mm. Smout's 6 weeks embryo, 33.1.5. Carpal region. The shaft of the ulna (*ul.*) is well formed and distinct from the muscles on each side, and the carpal region is formed by a dense mass of blastema in which the carpal elements are appearing as regions of greater density (*cp.*). The metacarpal region shows early centres of chondrification (*mt.*), but passes without any break into the blastema for the phalangeal region (*ph.*) and the dense tissues destined to form the flexor and extensor apparatus.

Fig. 7. 13 mm. Boyd's 'H 23', 24.1.5. Shoulder joint. The scapula with its characteristic blade (*bl.*) and coracoid (*cr.*) is well chondrified, and is separated from the head of the humerus (*hm.*) by an interzone (*i.z.*). The interzone is continuous at its margins with the perichondrium (*p.c.*) of the scapula and humerus, and through this with the very dense tissues surrounding the head of the humerus in which the tendons of the short muscles of the joint and the long head of the biceps will differentiate. The muscular belly of the deltoid (*dl.*) and its nerve supply (*n.s.*) are quite distinct.

Fig. 8. 13 mm. Boyd's 'H 23', 23.3.4. Humero-ulnar joint. The trochlear region of the humerus (*hm.*) is separated from the ulnar (*ul.*) by a thick interzone (*i.z.*). The ulna has not yet developed its olecranon and coronoid processes, though the triceps (*tr.*) is quite distinct, and there is little indication of the future shape of the joint surfaces.

Fig. 9. 13 mm. Boyd's 'H 23', 24.1.6. Carpus. The lower end of the radius (*r.d.*) is separated from the metacarpal region (*mt.*) by two masses of condensed blastemal tissue, which represent the proximal and distal rows of carpal elements (*cp.*). Between these masses the tissue is rarefied, and not condensed as yet to form typical interzones.

Fig. 10. 13 mm. Boyd's 'H 23', 40.2.4. Lower limb. The femur is now well defined and the lower end fully chondrified. The hip bone is chondrified and separated from the femur by an interzone. The tibia is well developed and the interzone at the knee has become sharply defined. The quadriceps muscle mass (*qu.*) surrounds the femoral nerve (*f.n.*), and the quadriceps tendon (*q.t.*) is seen passing in front of the knee joint region, quite distinct from it. The hamstring mass (*hm.*) surrounds the sciatic nerve (*s.n.*) in a similar way, and is also distinct from the skeletal tissues, being separated by a layer of connective tissue (*c.t.*). In the foot chondrification has not yet begun. In the tarsal region the elements are represented by dense masses of blastemal tissue (*ts.*). The metatarsal and phalangeal regions of the blastema (*mt.* and *ph.*) are not yet differentiated.

Fig. 11. 14 mm. Boyd's 'H 8', 19.2.8. Humero-ulnar joint. The trochlear surface of the humerus is separated from the sigmoid notch of the ulna by a well-defined interzone (*i.z.*) which follows the shape of the articular surfaces. The olecranon (*ol.*) and coronoid (*cr.*) processes of the ulna are now distinct. The interzone, perichondrium and tissues concerned with the tendons near the joint are still intimately blended.

Fig. 12. 14 mm. Boyd's 'H 8', 19.3.8. Fore-arm and hand. The carpal elements of the proximal and distal rows (*cp.*) are now chondrified, and are separated from each other and from the radius (*rd.*) and metacarpals (*mt.*) by typical interzones. The phalangeal region of the blastema (*ph.*) is still undifferentiated.

Fig. 13. 16 mm. Kirk's 'Series 1', 22.2.6. Shoulder. The interzone passes peripherally into the dense tissue (*d.t.*) that will form the capsule, labrum and neighbouring tendons, but these structures are not yet differentiated. Small blood vessels (*b.v.*) lie outside the perichondrium, forming a network which follows the shape of the cartilages, but none enters the perichondrium itself.

Fig. 14. 16 mm. Kirk's 'Series 1', 23.4.3. Humero-radial joint. Anteriorly and posteriorly the fibrous capsule (*f.c.*) arches over the joint region, enclosing the synovial mesachyme (*s.m.*) between its inner surface and the intra-capsular perichondrium (*i.p.*). Small blood vessels lie within the capsule.

PLATE 2

Fig. 15. 16 mm. Kirk's 'Series I', 23.4.5. Metacarpo-phalangeal region. The metacarpal (*mt.*) is well chondrified and proportionately large, the basal phalanx (*b.p.*) has now chondrified, but beyond this phalanx the blastema (*bl.*) is undifferentiated. The joint region is surrounded by mesenchymal tissue in which the capsule has not yet appeared.

Fig. 16. 16 mm. Kirk's 'Series I', 41.1.6. Knee. The capsule (*cp.*) is well developed posteriorly and cuts off an abundant synovial mesenchyme (*s.m.*) with its included blood vessels (*b.v.*).

Fig. 17. 19 mm. Appleton's 'H 19', 4.3.6. Humero-radial joint. The homogeneous interzone (*i.z.*), intra- and extra-capsular perichondrium (*i.p.* and *e.p.*), synovial mesenchyme (*s.m.*) and fibrous capsule (*cp.*) take the classical form.

Fig. 18. 19 mm. Appleton's 'H 19', 3.3.5. Carpus. The carpal elements, the trapezium (*tm.*), trapezoid (*td.*), scaphoid (*sc.*) and centrale (*ce.*), are separated from each other and from the radius and metacarpals by interzones of the typical form. The capsule of the wrist joint (*cp.*) can be distinguished enclosing the synovial mesenchyme (*s.m.*).

Fig. 19. 19 mm. Appleton's 'H 19', 3.2.3. Carpo-metacarpal and metacarpo phalangeal joints of the thumb. The fibrous capsules (*cp.*) are dense in these joints, for the tissue destined to form the collateral ligaments is not yet differentiated from them. The bulging curves of the capsule of the metacarpo-phalangeal joint are very clearly shown, uncomplicated by associated condensed structures.

Fig. 20. 21 mm. Kirk's 'Series 8', 78.1.2. Shoulder. A loose zone (*l.z.*) separating two dense chondrogenous layers (*c.l.*) can barely be distinguished in the interzone. The capsule (*cp.*) is now distinct and encloses a scanty synovial mesenchyme (*s.m.*).

Fig. 21. 21 mm. Kirk's 'Series 8', 90.1.3. Elbow. The three-layered interzone with its chondrogenous layers (*c.l.*) and loose intermediate layer (*i.l.*) is shown fully developed between humerus and radius and partly developed between humerus and ulna. The chondrogenous layers are continued into the intra-capsular perichondrium (*i.p.*) and the intermediate layer into the synovial mesenchyme (*s.m.*) which lies within the capsule (*cp.*).

Fig. 22. 21 mm. Kirk's 'Series 8', 85.1.3. Knee. The condyles of the femur are separated from the tibia by homogeneous interzones (*i.z.*), and the intercondylar space is filled by synovial mesenchyme (*s.m.*). The menisci have not yet appeared. Between the femur and the quadriceps tendon is a layer of loose synovial mesenchyme (*l.m.*) where the joint cavity will appear at a later stage.

Fig. 23. 23 mm. Appleton's 'H 23', 8.2.2. Shoulder. A typical three-layered interzone has appeared at this stage. The chondrogenous layer (*c.l.*) covers the glenoid surface of the scapula and is continued into the labrum glenoidale (*l.g.*).

Fig. 24. 24 mm. London Hospital 'mx' (Rutherford), 120.12. Shoulder. The labrum glenoidale is very distinct and is continuous with the chondrogenous layer of the interzone.

Fig. 25. 26 mm. Boyd's 'H 11', 75.2.1. Knee, condylar region. The interzone between the condyle of the femur and tibia is in the early three-layered stage. The menisci (*mn.*) are indicated by faint condensation of the mesenchyme.

Fig. 26. 26 mm. Boyd's 'H 11', 74.1.3. Knee, intercondylar region. In the abundant synovial mesenchyme (*s.m.*), which contains numerous small blood vessels, is the posterior cruciate ligament (*cr.*). The patella is separated from the femur by a layer of loose synovial mesenchyme (*l.m.*).

Fig. 27. 26 mm. Boyd's 'H 11', 75.2.1. Tarsus. The talus (*ta.*), scaphoid (*sc.*), first cuneiform (*cn.*) and metatarsal are separated by homogeneous interzones.

Fig. 28. 29 mm. Appleton's 'H 29', 10.2.2. Shoulder. Liquefaction of the synovial mesenchyme has begun at the periphery of the joint, so that the position of the joint cavity (*j.c.*) is clearly defined, but the cavity is still partly filled with cells. The intermediate layer of the interzone (*i.l.*) appears collapsed, and its flattened cells are clumped, but this condition is probably an artifact caused by swelling of the cartilage. A pressure fold (*p.f.*) indicates swelling of the cartilage at some stage in the preparation.

Fig. 29. 29 mm. Appleton's 'H 29', 26.2.1. Humero-ulnar joint. The three layers of the interzone are sharply defined and in the intermediate layer the cells have become flattened and arranged parallel to the articular surfaces.

Fig. 30. 29 mm. Appleton's 'H 29', 28.2.5. Fourth metacarpo-phalangeal joint. The interzone is in the early three-layered stage.

Fig. 31. 30 mm. Appleton's 'H 30', 12.2.7. Shoulder. In the peripheral parts of the joint the synovial mesenchyme is now liquefied to form the joint cavity (*j.c.*). More centrally the scapula and humerus appear to be in continuity, but this is probably due to an artifact, as in Fig. 28.

PLATE 3

Fig. 32. 30 mm. Appleton's 'H 30', 5.8. Humero-radial joint. The middle layer of the interzone is liquefying and a small joint cavity has appeared anteriorly. The chondrogenic layers of the interzone (*c.l.*) are still recognizable. The surface of the synovial mesenchyme is ragged. Loose tissue (*l.t.*) separates the annular ligament (*a.l.*) from the head of the radius.

Fig. 33. 30 mm. Appleton's 'H 30', 10.3. Humero-ulnar joint. This section is from the same series as that shown in Fig. 32, and probably had a three-layered interzone, but as a result of pressure the interzone appears as a single dense layer of cells.

Fig. 34. 30 mm. Appleton's 'H 30'. Carpal region. Most of the interzones are three layered in structure. The homogeneous appearance of some of the interzones (*h.*) is probably due to artifact.

Fig. 35. West's 34 mm. embryo, 12.2.4. Humero-radial joint. The humerus and radius are separated by a synovial cavity which still contains some scattered strands of cells (*s.c.*). Between the head of the radius and the annular ligament (*a.l.*) is a layer of loose mesenchyme (*l.m.*) which will later break down to form a part of the joint cavity.

Fig. 36. West's 34 mm. embryo, 10.1.4. Humero-ulnar joint. Near the coronoid process the interzone has a typical three-layered structure, with a well-developed intermediate layer (*i.l.*). More posteriorly the intermediate layer appears as if compressed, an appearance probably due to artifact.

Fig. 37. West's 34 mm. embryo, 10.1.3. Proximal inter-phalangeal joint of the fourth digit of the hand. The interzone is in a typical three-layered stage, with distinct chondrogenic layers (*c.l.*), and a loose intermediate layer (*i.l.*). This stage of the interzone has not usually been recognized in the inter-phalangeal joint.

Fig. 38. West's 34 mm. embryo, 20.1.5. Hip-joint. The head of the femur is seen in acetabulum. The ligamentum teres (*l.t.*) lies in a mass of synovial mesenchyme (*s.m.*) accompanied by conspicuous blood vessels (*b.v.*).

Fig. 39. West's 34 mm. embryo, 9.2.4. Knee. A small joint cavity has developed between the meniscus and the condyle of the femur, but the meniscus is still attached to the tibia by synovial mesenchyme.

Fig. 40. West's 34 mm. embryo, 6.2.5. Knee. A joint cavity (*j.c.*) is forming between the patella and femur. The anterior cruciate ligament (*cr.*) lies in a mass of synovial mesenchyme well supplied with blood vessels (*b.v.*).

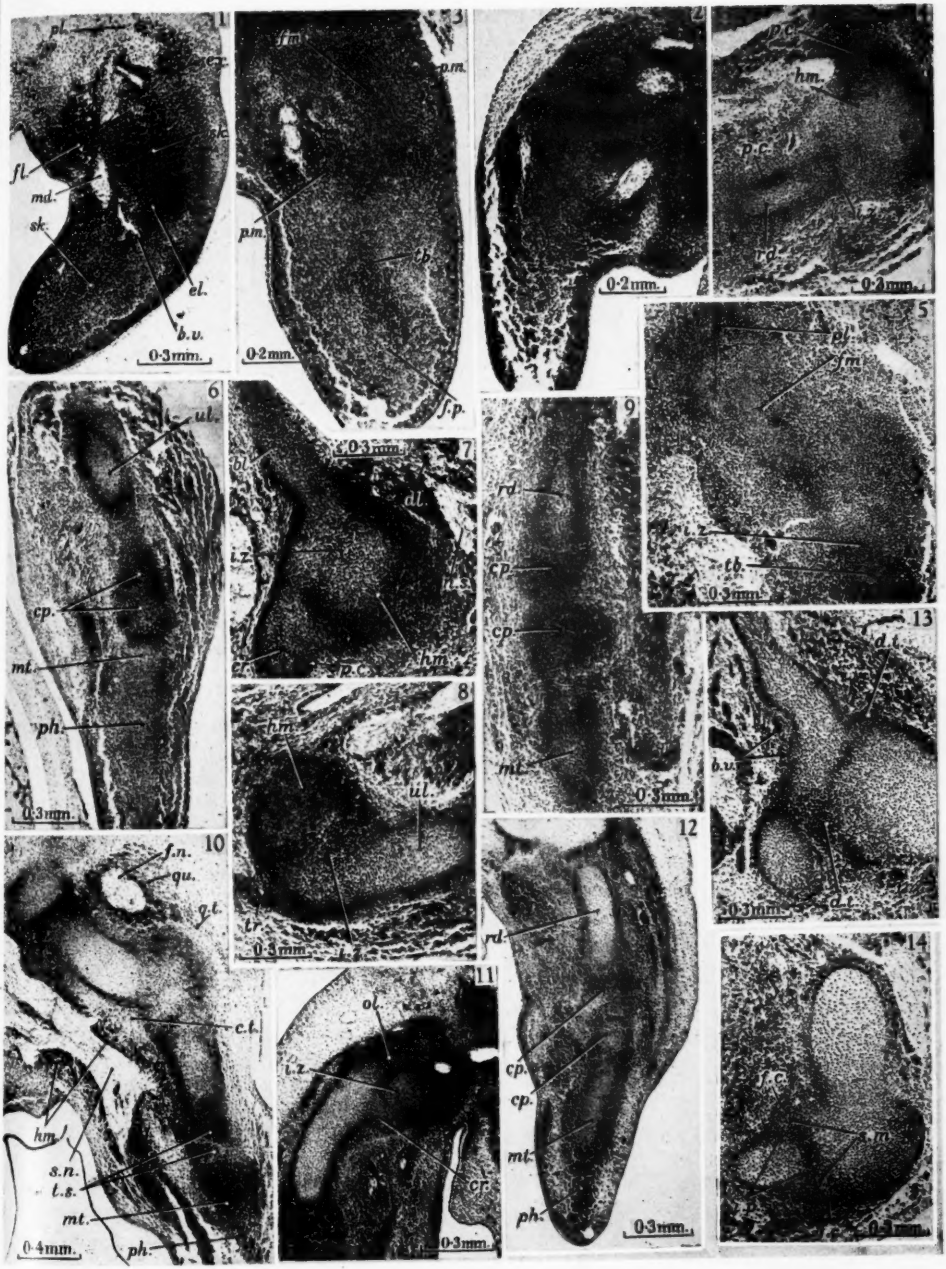
Fig. 41. West's 34 mm. embryo, 4.1.6. Knee. The tendon of the popliteus (*pp.*) is seen in its characteristic position above the head of the fibula and outside the meniscus. The sesamoid in the lateral head of the gastrocnemius (*sm.*) is in the pro-cartilaginous stage. A small joint cavity (*j.c.*) is liquefying between the sesamoid and the femoral condyle.

Fig. 42. West's 34 mm. embryo, 9.1.4. Ankle. The interzones of the ankle and intertarsal joints are all in the three-layered stage. The synovial sheaths (*s.s.*) of the peroneus longus and Achilles' tendon are already developed.

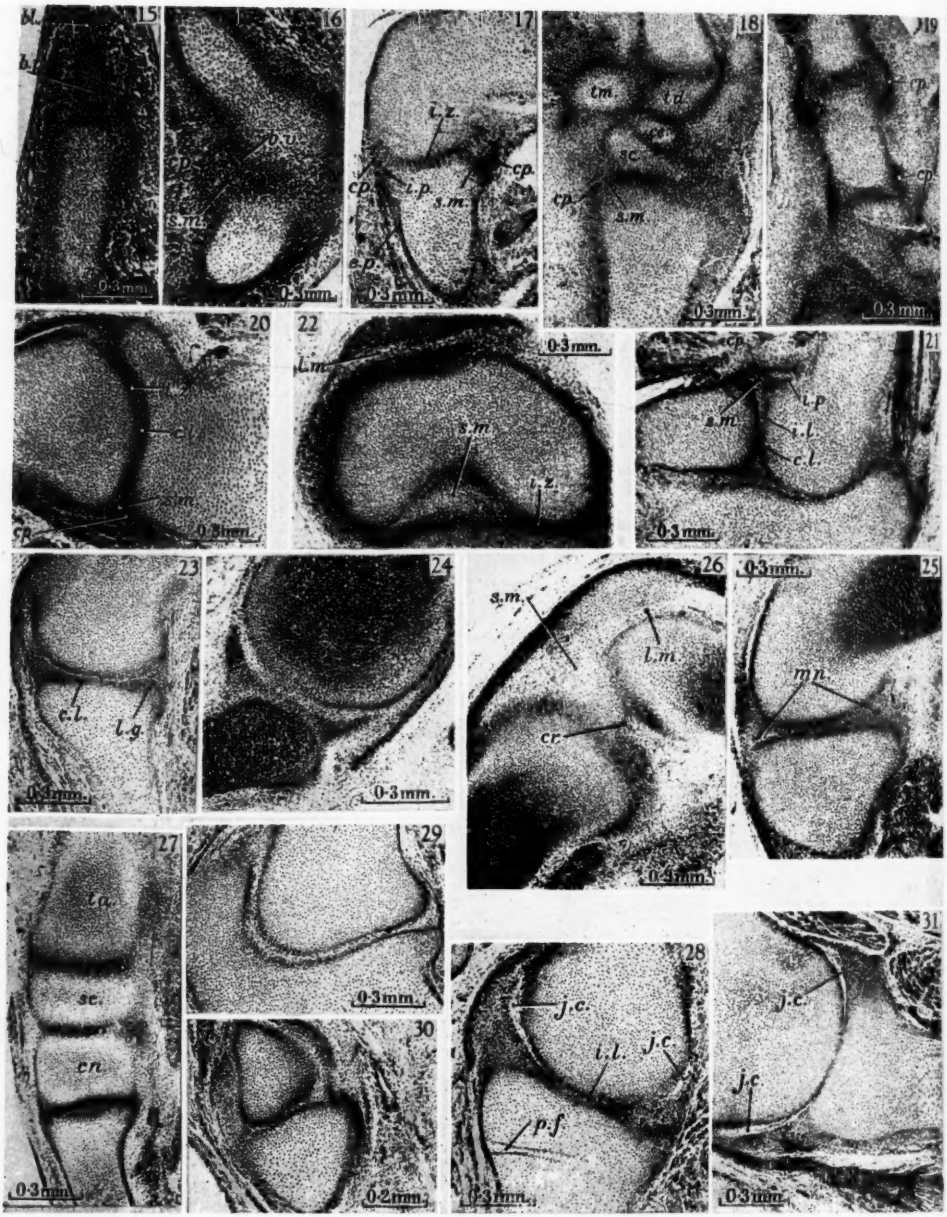
Fig. 43. 45 mm. Appleton's 'H 45', 4.2. Humero-radial joint. The humerus and radius appear joined, but this is probably an artifact. The joint cavity (*j.c.*) between the annular ligament (*a.l.*) and the head of the radius is well developed. Synovial folds (*s.f.*) project into the joint, and are covered by smooth strata of synovial cells (*s.c.*).

Fig. 44. 45 mm. Appleton's 'H 45', 4.2. Humero-radial joint. High-power view of the articular surfaces from the section illustrated in Fig. 43. The surfaces are covered by fibrillar layers (*f.l.*), which unite where the surfaces join. Flat cells (*f.c.*) lie in and near these layers, and some cellular debris (*d.*) can be detected.

Fig. 45. 45 mm. Appleton's 'H 45', 26.1. Knee-joint. The menisci are free from the femur but still joined to the tibia by loose synovial mesenchyme.



WHEELER HAINES—THE DEVELOPMENT OF JOINTS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0·3mm.
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s.m.
cp.
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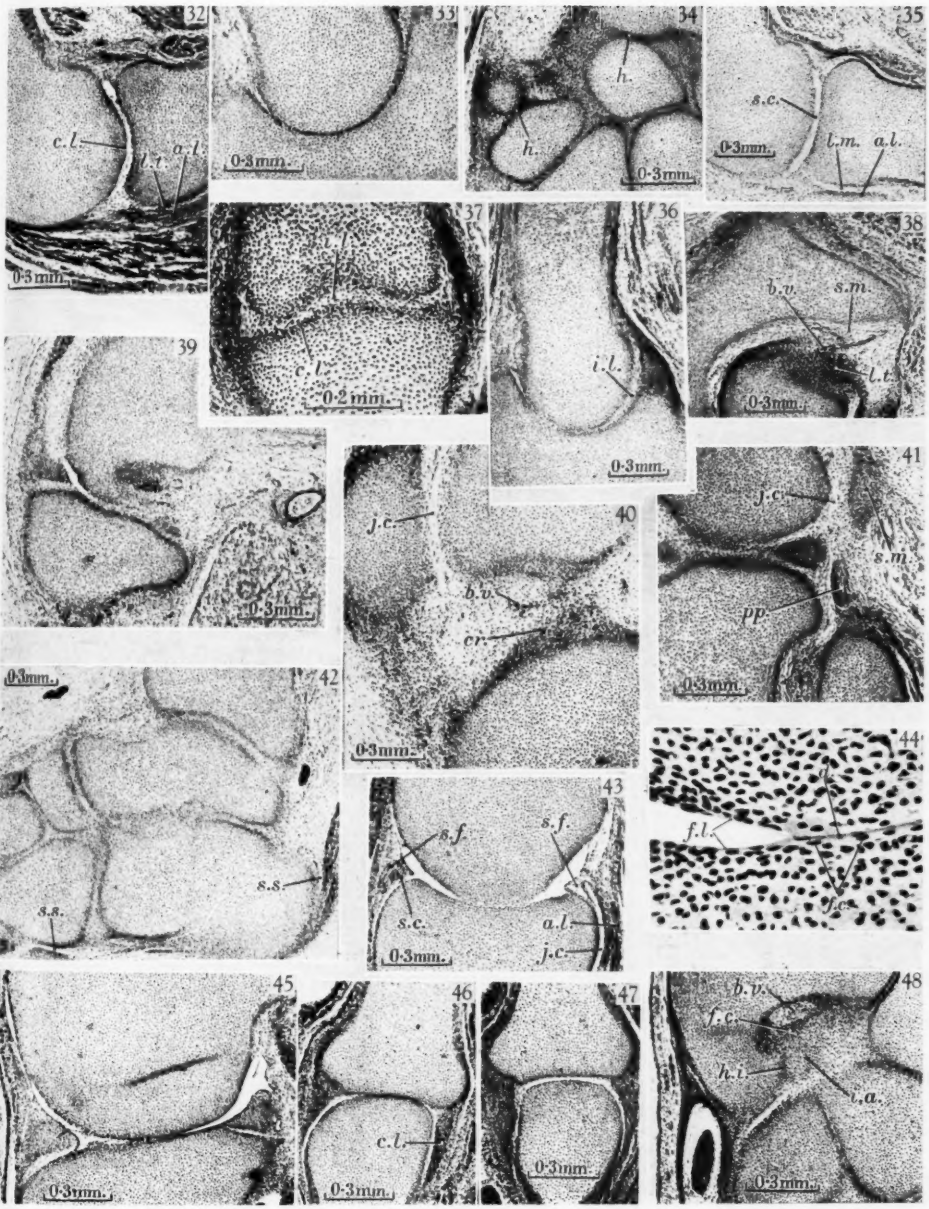
32
c.l.
l.t.
a.l.
0·5 mm.
33
0·3 mm.
34
h.
h.
0·3 mm.
35
s.c.
l.m.
a.l.
0·3 mm.
37
c.l.
c.l.
0·2 mm.
36
i.l.
0·3 mm.
38
b.v.
s.m.
l.t.
0·3 mm.
39
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40
j.c.
b.v.
cr.
0·3 mm.
41
j.c.
s.m.
pp.
0·3 mm.
0·3 mm.
42
s.s.
s.s.
43
s.f.
s.f.
s.c.
a.l.
j.c.
0·3 mm.
44
f.l.
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46
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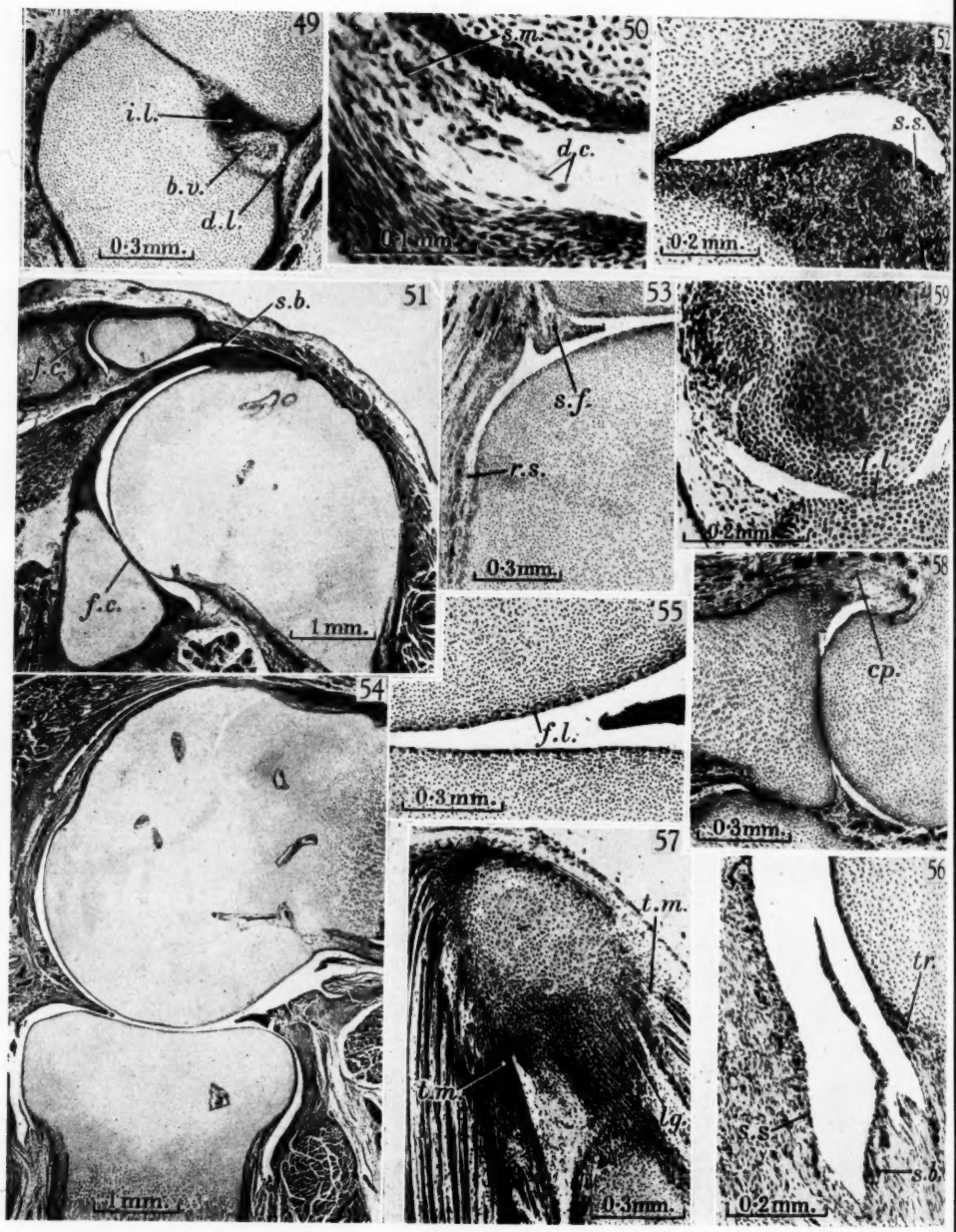
48
b.v.
f.c.
h.l.
c.a.
0·3 mm.


49
i.l.
b.v.
d.l.
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50
s.m.
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0·1mm.
52
s.s.
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51
s.b.
f.c.
f.c.
1mm.
53
s.f.
r.s.
0·3mm.
59
f.l.
0·2mm.
58
cp.
0·3mm.
55
f.l.
0·3mm.
54
1mm.
57
t.m.
t.m.
lg.
0·3mm.
56
tr.
s.s.
s.b.
0·2mm.

Fig. 46. 45 mm. Appleton's 'H 45', 4.2. Metacarpo-phalangeal joint of the third digit of the hand. The cartilages appear joined. The collateral ligaments (*c.l.*) are seen on either side.

Fig. 47. 45 mm. Appleton's 'H 45', 10.6. Metacarpo-phalangeal joint of the fifth digit of the hand. In this section from the same series as Fig. 46, the cartilages appear separate.

Fig. 48. 49 mm. Appleton's 'H 49', 33.1. Wrist. The fibro-cartilage (*f.c.*) of the wrist is found as a condensation of the synovial mesenchyme, with blood vessels (*b.v.*) nearby. A cartilage, the 'intermedium antibrachii' (*i.a.*), is found at this stage of development separated from the styloid process of the ulna by a typical homogeneous interzone (*h.i.*).

PLATE 4

Fig. 49. Hughes's 60 mm. embryo, 7.3.8. Intercarpal joints between capitate and trapezoid. The interosseous (*i.l.*) and dorsal (*d.l.*) ligaments enclose between them a mass of synovial mesenchyme containing blood vessels (*b.v.*).

Fig. 50. Hughes's 60 mm. embryo, 3.3.7. Inferior radio-ulnar joint. The joint cavity is spreading by liquefaction of the synovial mesenchyme (*s.m.*). The synovial surface is ragged, and strands of cells stretch into the cavity. Some of the cells are degenerate in appearance (*d.c.*).

Fig. 51. 66 mm. Appleton's 'H 66', 23.1. Shoulder and acromio-clavicular joint. The synovial surfaces are smooth and the humeral head is more curved than the glenoid surface. The walls of the sub-acromial bursa (*s.b.*) have a structure similar to those of the joint cavity. The clavicle and glenoid surface are covered with layers of articular fibro-cartilage (*f.c.*).

Fig. 52. 66 mm. Appleton's 'H 66', 24.3. Shoulder. The recess of the joint cavity showing the synovial stratum (*s.s.*), and the vascular sub-synovial tissue.

Fig. 53. 125 mm. Appleton's 'H 125', 18.1. Proximal inter-phalangeal joint of the third digit of the hand, illustrating the formation of a synovial fold (*s.f.*). The ragged synovial surface (*r.s.*) indicates that the joint cavity is still extending by liquefaction of the synovial mesenchyme.

Fig. 54. 190 mm. Appleton's 'H 190', 2.3. Humero-radial joint. General view showing the incongruity of the articular surfaces, the synovial folds and synovial villi.

Fig. 55. 190 mm. Appleton's 'H 190', 2.3. Same section. The articular surfaces of the cartilages show distinct fibrillar layers (*f.l.*), and the structure of the cartilage has not attained its adult pattern.

Fig. 56. 190 mm. Appleton's 'H 190', 2.3. Same section. High-power view of a synovial villus, showing also the synovial stratum (*s.s.*), a poorly developed sub-synovial stratum (*s.b.*), and a typical transition zone (*tr.*) between the articular cartilage and perichondrial tissues.

Fig. 57. Hughes's 20 day rat. The radial collateral ligament (*lg.*) shows twisting of its fibres and a structural similarity to the tendons of origin of the extensor muscles (*t.m.*).

Fig. 58. Hughes's 20 day rat. Humero-radial joint. General view showing the apparent union of the cartilages, and the structure of the fibrous capsule (*cp.*).

Fig. 59. Hughes's 20 day rat. Metacarpo-phalangeal joint of second digit. The cartilages are apparently joined, but in the region of union the fibrillar layer (*f.l.*) is conspicuous.

THE GROWTH AND MATURATION OF REGENERATING STRIATED MUSCLE FIBRES

By W. E. LE GROS CLARK and H. S. WAJDA
Department of Anatomy, University of Oxford

In previous papers (Le Gros Clark & Blomfield, 1945; Le Gros Clark, 1946), an account has been given of the process of regeneration which occurs in the tibialis anterior muscle of the rabbit following ischaemic necrosis. Necrosis was induced by interrupting the main vessels of supply, an operation which leads to an effective ischaemia involving approximately the lower two-thirds of the muscle. In this region, most of the muscle fibres undergo necrosis, though a variable number persist alongside the main anastomotic vascular channels and also some at the surface of the muscle in relation to the epimysium (as well as a few close to the tendon of insertion). The necrotic tissue is rapidly removed by macrophage activity and is soon replaced by the outgrowth of fine sarcoplasmic strands from the stumps of the old muscle fibres which remain in the upper third of the muscle. Under favourable conditions, it was found that the regenerated fibres may develop into fibres of mature appearance and that the necrotic part of the muscle may even come to assume a practically normal structure. Among other observations, it was also estimated that the rate of longitudinal growth of regenerating muscle fibre may reach a maximum of at least 1·5 mm. a day.

Further experiments have now been carried out in amplification of those already reported, with special reference to the rate of growth in length and width of regenerating muscle fibres, and the effect of immobilization and tenotomy on the course of regeneration.

THE RATE OF GROWTH OF REGENERATING MUSCLE FIBRES

The estimation of the rate of growth of regenerating muscle fibres presents certain practical difficulties, and it is clear that a large amount of experimental material is needed to permit of results of more than approximate value. For example, our material indicates that the rate of growth in length and diameter varies to some extent even in muscles which appear superficially to have received similar degrees of injury. Thus it is probable that the growth rate is influenced by secondary factors such, perhaps, as the intensity of the initial inflammatory reaction, the functional activity of the limb during the convalescent period and the age of the animal. The variability of the first two factors is not easy to control. It must be emphasized, also, that the extent of the necrosis in the lower part of the tibialis anterior muscle which follows interruption of the main vessels of supply varies from one experiment to

another, depending no doubt on individual variation in the size and number of intramuscular anastomotic channels. In our experience, however, most of the muscular tissue in the lower portion of the muscle undergoes necrosis, with the exceptions already mentioned.

In measuring the length of new fibres, the end-points between which the measurement is made can usually be determined fairly clearly from sections taken through the long axis of the muscle. One point is the abrupt transition between the stumps of old surviving fibres and the slender outgrowing young fibres, marked usually by an abrupt change in size and cytological characters and by a difference in staining properties. The other point is represented by the 'spear heads' of the young regenerating fibres. However, it is seldom possible to follow in a single section one individual new fibre throughout its whole extent, for it is only rarely that a fibre follows a sufficiently straight course. It has, therefore, been necessary to estimate the mean length of newly growing fibres from a number of measurements made on several sections of each specimen. In some of the experimental material this could be done with considerable accuracy since the area of necrosis and repair was demarcated from the healthy tissue by a relatively sharply defined and even line. Altogether sections (partly serial) of twenty-two devascularized muscles were available, covering a wide range from the earliest stages of repair up to 4 months after devascularization. However, for a study of the rate of growth in length, muscles removed more than 2 weeks after devascularization were useless since by this time some of the regenerating fibres had extended throughout the whole of the necrotic zone. For this part of the study, tibialis anterior muscles from ten experiments were available.

During the first 4 days after devascularization, the growth of new fibres in the tibialis anterior has not proceeded far enough to allow of direct measurements of even approximate accuracy. This initial delay is evidently due to the fact that rapid growth is not possible until the removal by phagocytic activity of the old disintegrating muscle fibres at the margin of the necrotic area has yielded some free space. By the fifth day, however, the average of measurements made on a number of different sections in each of two experiments amounts to 6·5 mm. Thereafter, up to 14 days, growth proceeds at a steady rate as indicated in the graph shown in Fig. 1. In this graph each point is based on the average measurements of the maximum lengths of regenerating fibres made in a number of sections in each of two separate experiments, with the exception of the 11-day interval which is based on one experiment only.

From the graph it appears that the average rate of growth is of the order of 1·2 mm. a day from the 4th to the 14th day. Maximum measurements taken on individual sections, however, indicate that it is possible for the rate to reach as much as 1·7 mm. a day.

The estimation of the rate of growth in width of regenerating muscle fibres presented fewer technical difficulties. The young fibres at different stages of maturation show an even calibre except for the terminal expansions at their

growing tips. Those regenerating fibres were measured which showed the most advanced degree of differentiation at different intervals after devascularization. In each experiment, such measurements were made directly with an ocular micrometer on a number of serial sections. The number of experiments on which each average is based was as follows:

2 days' stage	3 experiments	11 days' stage	1 experiment
4 days' stage	3 „	14 days' stage	2 experiments
5 days' stage	2 „	21 days' stage	3 „
8 days' stage	2 „	4 months' stage	6 „

In the six long-term experiments (4 months after devascularization) transverse sections were prepared through each tibialis anterior muscle in the middle of its upper third and through the middle of its lower (regenerated) half.

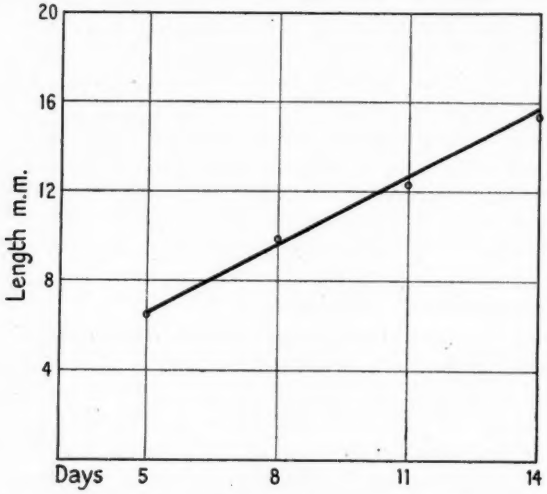

Fig. 1. Graph showing the rate of growth in length of regenerating muscle fibres during the first 2 weeks after inducing ischaemic necrosis.

Equivalent sections were made in each case at the same levels through the normal tibialis anterior of the opposite side. In measuring the diameter of muscle fibres in this material, those near the surface were ignored since here they are not affected by the presence of surrounding fibres and their cross-sectional shape tends often to be somewhat irregular.

In the material from the earlier experiments (up to 21 days after devascularization), it was always possible to distinguish the young regenerating fibres from those original fibres which might have survived in the ischaemic part of the muscle. In the long-term experiments such a distinction could no longer be made and in these cases the measurements must have included a variable

proportion of the original surviving fibres. For this reason, the figures given for the average width of regenerated fibres in the muscles examined 4 months after devascularization may be too high. On the other hand, a comparison of the distribution of fibre size in the upper and lower parts of the devascularized muscles gives no reason for supposing that there is a significant discrepancy here.

In the graph, shown in Fig. 2, the rate of growth in width of regenerating muscle fibres is shown. The mean width of fibres in the normal tibialis anterior (based on the measurement of 240 fibres) was found to be 50·1 μ; the difference

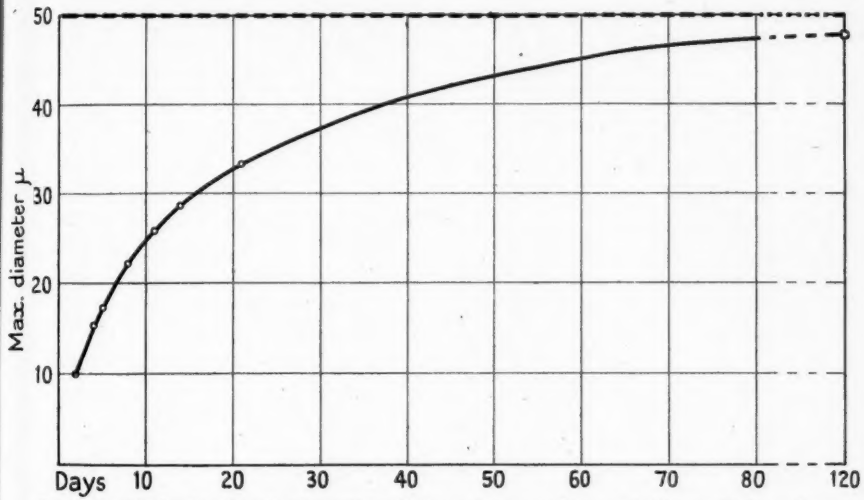

Fig. 2. Graph showing the rate of growth in thickness of regenerating muscle fibres up to 4 months after inducing ischaemic necrosis.

between the upper and lower part of the normal muscle was insignificant (50·2 and 50·0 μ). Four months after devascularization, the mean diameter of fibres in the lower (regenerated) portion of the tibialis anterior was 48·3 μ, i.e. 97 % of the normal value. It is interesting to note that the fibres in the upper (unaffected) part of the devascularized muscles showed a slight degree of hypertrophy, for in each of the six experiments their average diameter was consistently greater than normal. The total average amounted to 54·3 μ, i.e. 108 % of the normal value. This hypertrophy is no doubt to be regarded as compensatory in respect of the functional deficiency of the lower part of the muscle.

THE FUNCTIONAL EFFICIENCY OF THE TIBIALIS ANTERIOR MUSCLE FOLLOWING REPAIR

In the six long-term experiments referred to above, in which the muscles were removed 4 months after devascularization, the results as evidenced by naked-eye

inspection were variable, and in most cases a marked degree of fibrosis had obviously supervened. The fibrosis, however, was partly superficial, involving a thickening of the epimysium. It is evidently for this reason that in all but one of the experiments the devascularized muscle was found to be heavier than the normal muscle of the opposite side. The weights (in grams) of the freshly removed muscles were as follows:

	Weight of devascularized muscle	Weight of normal muscle
	g.	g.
R375	3·7	3·1
R376	4·6	4·5
R377	4·4	3·9
R378	3·5	3·5
R379	4·9	3·9
R380	3·8	3·4

The functional efficiency of the devascularized muscles was in each case tested before its removal and compared with that of the opposite side. For this purpose a thread was attached to the tendon of the tibialis anterior and passed over a pulley to a balance tray. The latter was then loaded up to the point where an approximately isometric contraction resulted from the electrical stimulation of the lateral popliteal nerve. The tensions developed ranged up to 1600 g. The results of these experiments (which, of course, can only give approximate results) showed no marked difference in the maximum isometric tensions which developed in the normal as compared with the devascularized muscles. Visual observation, also, showed no obvious difference in the response to stimulation of the upper and lower parts of the devascularized muscle, the latter contracting apparently uniformly throughout its extent. As already indicated, the functional efficiency in isometric contraction of the devascularized muscle is partly to be accounted for by the hypertrophy of the fibres in the upper, unaffected, part of the muscle.

INFLUENCE OF IMMOBILIZATION ON THE REPAIR PROCESSES IN DEVASCULARIZATION

For the purpose of studying the influence of movement on the course of muscle regeneration, the tibialis anterior muscle in three rabbits was devascularized on both sides and the right extremity, including the ankle and the knee joint, immobilized in a plaster of paris casing. Care was taken as far as possible to avoid interference with the circulation of the limb by undue pressure.

The animals were killed 10 days, 13 days and 2 months respectively after the operation, and the muscles of both sides excised and prepared for histological examination. In the 10-days' specimen the muscle of the immobilized extremity was found to be completely divided into two widely separated portions, the lower portion corresponding to the area of devascularization. Macroscopically it presented the appearance of grossly inflamed tissue with

numerous patches of necrosis on its surface. Microscopically it was found to contain muscle fibres in all stages of degeneration up to complete necrosis. Hyalinized fibres devoid of nuclei prevail towards the centre of the muscle. More peripherally the fibres are in process of discoidal disintegration, and are separated one from another by a considerable amount of nuclear debris and great numbers of polymorphonuclears. In contrast with the severe degree of tissue necrosis in the immobilized tibialis anterior, that from the opposite (free) side showed a process of repair and regeneration comparable to that which has previously been described in the devascularized muscle. Somewhat more favourable conditions were found in the 13-day specimen, though it was evident already at autopsy that the immobilized muscle had been greatly impaired, and histological examination showed that even the upper portion, usually unaffected by devascularization, was far from being normal, many of the muscle fibres being fragmented and in process of hyalinization. At the same time, especially near the surface of the muscle and along the main vessels of supply, new muscle fibres are seen growing in limited numbers. The lower portion of muscle, corresponding to the area of almost complete devascularization, is demarcated from the upper part by a zone of erratically growing fibres. This zone, particularly rich in instances of secondary degeneration (i.e. degeneration of regenerated muscular tissue) shows a considerable amount of fibrosis. Patches of fatty infiltration are conspicuous throughout the lower third of the muscle. The main bulk of this part of the muscle, however, is composed of young regenerating muscle fibres which are already approaching an almost mature stage. They are richly nucleated and the amount of interstitial connective tissue is higher than normal. In the tibialis anterior of the opposite (free) side, regenerating muscle fibres in the lower part of the muscle are far more numerous and also more regularly disposed.

In the muscle which had been devascularized and immobilized for 2 months, the following histological details are to be observed. The upper third is composed of fibres which appear normal except that they are more richly nucleated than usual, and in some places rows of closely packed nuclei indicate their recent amitotic proliferation. Distally the fibres become more irregular, and the muscle shows an interstitial fibrosis and a considerable degree of fatty infiltration. In the lower two-thirds of the muscle the most striking feature is the fineness of the young muscle fibres which have replaced the old degenerated fibres, but in some places these young fibres show in turn the characteristic pictures of muscle degeneration. In spite of these degenerative changes there is very little, if any, inflammatory cellular reaction. In the devascularized and non-immobilized tibialis anterior of the opposite side, there is no fatty infiltration of the muscle and no secondary degeneration of the regenerating fibres. As in the immobilized muscle, there is some degree of fibrosis, but it is confined chiefly to the epimysium rather than the endomysium.

The results of these three experiments show that the processes of repair and regeneration in the devascularized anterior tibial muscle were severely

impaired in the limbs which had been immobilized in plaster. In spite of the care taken to avoid pressure effects, it is perhaps premature to affirm (without further experiments) that the impairment was due to immobilization *per se* and not (at least in part) to interference with the blood supply to the limb.

REGENERATION IN TENOTOMIZED MUSCLES

In the rapid regenerative process which occurs in the rabbit's tibialis anterior muscle following ischaemic necrosis, it is possible that the tension exerted by the proximal, undamaged part of the muscle through the tendon of insertion may play a significant part, particularly in maintaining the alinement of the endomysial tubes which appear to facilitate and direct the outgrowth of regenerating fibres. In order to investigate this point, in two animals the tibialis anterior muscles of both sides were devascularized in the usual way and in each case the tendon of insertion of the muscle of the right side was cut. The animals were killed after intervals of 7 and 10 days. In both experiments, the repair process on the control (left) side corresponded closely in its extent and histological details with that previously described for similar time intervals. On the tenotomized side, regenerative activity appeared actually to be somewhat accelerated, for the removal of degenerated tissue and the growth of young fibres showed some advance in comparison with the control muscles. On the other hand, there was considerably more secondary degeneration on the tenotomized side, many of the newly formed young fibres showing pyknotic nuclei and hyaline degeneration of the contractile substance. Moreover, the regenerated fibres were found to be mingled with rather abundant fibroblastic tissue. Thus, while it may be inferred from these two experiments that tenotomy does not lead to any retardation in the process of regeneration (and, indeed, may even be associated with some degree of acceleration), it is clear that the early maturation of the regenerated muscular tissue is adversely affected.

SUMMARY

1. The average rate of growth in length of regenerating striated muscle fibres under favourable conditions is of the order of 1·2 mm. a day from the 4th to the 14th day. In some cases this rate may reach as much as 1·7 mm. a day.

2. The growth in diameter of regenerating muscle fibres proceeds rapidly at first and by 21 days the average width of the young fibre is two-thirds that of the normal fibre. After 4 months, the mean diameter of the fibre in the devascularized portion of the tibialis anterior was found to be 97 % of the normal value.

3. Four months after devascularization, the fibres in the upper, unaffected part of the tibialis anterior show some hypertrophy.

4. In three experiments in which the limb had been immobilized after devascularization of the tibialis anterior, the processes of repair and regeneration were found to be severely impaired.

5. The initial stages of regeneration of muscle fibres are not retarded by tenotomy of the tibialis anterior, but the newly formed fibres show more histological evidence of secondary degeneration than on the control side, and they are mingled with a greater abundance of fibroblastic tissue.

REFERENCES

Le Gros Clark, W. E. (1946). The regeneration of mammalian striated muscle. *J. Anat., Lond.*, **80**, 24–36.

Le Gros Clark, W. E. & Blomfield, L. B. (1945). The efficiency of intramuscular anastomoses, with observations on the regeneration of devascularized muscle. *J. Anat., Lond.*, **79**, 15–33.

THE PRENATAL MEDULLATION OF THE SHEEP'S NERVOUS SYSTEM

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INTRODUCTION

Many attempts have been made to correlate the appearance of stainable myelin in the nervous system with the onset of function in developing tracts and nerves (Tilney & Casamajor, 1924; Langworthy, 1926, 1928 *a*, *b*, 1930, 1933; Angulo y Gonzalez, 1929). Langworthy (1933) gives a good review of the literature and comes to the conclusion that medullation occurs in different fibre systems in the order of their phylogenetic development, and that normal function and medullation are closely correlated in their time of onset.

The sheep foetus is considered a suitable subject for the study of medullation in the nervous system because, unlike most of the animals which have been investigated, it is born in an advanced state of development and the various stages in the functional growth of the nervous system are well known (Barcroft & Barron, 1936, 1937, 1939, 1942).

MATERIAL AND METHODS

Sheep foetuses ranging in age from 53 to 140 days after insemination have been used (Table 1). With the exception of two of these (53- and 63-day-old foetuses) which are aged on the basis of their crown-rump length and weight, all have been obtained by Caesarean section from sheep whose stage of gestation is

Table 1. *Sheep foetuses*

No.	Age in days	Weight in g.	C.R.L. in mm.
—	53	25·5	80·0
—	63	102	142
299	66	97·2	140
340	78	222·2	180
268	78	229·0	192
281	84	348·2	210
331	96	533	250
309	111	1450	355
682	120	2200	390
689	129	1670	360
98	140	4090	470

accurately known. After fixation by injection with 4 % formaldehyde through an umbilical artery the brains were removed, cut into slices not more than 1 cm. in thickness and mordanted in Weigert's primary mordant (bichromate-fluorchrome) for 14 days at 30° C. The pieces were then thoroughly washed in water, dehydrated through absolute alcohol and embedded in either celloidin

or paraffin. The latter method gives good results, and where relatively thin sections (15 μ) are cut these can be affixed to albumenized slides without difficulty by floating on 70 % alcohol, blotting with cigarette paper and drying on the hot plate. Thin sections are infinitely preferable to thick ones for demonstrating early myelin, as the differentiation of the background without destaining the myelin is much simpler.

Sections were then placed for 1 hr. in Weigert's gliabieze, washed, stained for 4–6 hr. in Kultschitzky's haematoxylin at 37° C., rewashed, rinsed in gliabieze and differentiated by repeated short immersions in 0·25 % potassium permanganate followed by weak sulphurous acid.

With this method it has been found possible to show the finest myelin sheaths and at the same time obtain adequate differentiation of the background.

Serial sections throughout the whole brain and parts of the spinal cord have been cut in all but the 84-day-old foetus where only the spinal cord had been obtained.

OBSERVATIONS

In the following description no attempt has been made to ascertain the degree of medullation of individual tracts at every stage, but only the earliest time at which myelin is seen in each tract.

Table 2 gives a summary of the main observations, showing the earliest age at which myelin is seen. Where a trace (tr.) is marked on the table this means that only a very few fibres have a myelin sheath at this age. A + does not necessarily mean that every fibre is medullated but that the majority have some myelin.

No myelin is present in any part of the nervous system of the 53-day sheep foetus, though by 63 days several elements of the nervous system have begun to acquire myelin sheaths. The most obvious of these is the vestibular nerve whose branches can be traced into the lateral, superior and medial vestibular nuclei. A few fibres pass through the latter to decussate with similar fibres of the opposite side and appear to pass to the contralateral vestibular nuclei. The uncrossed vestibulo-spinal tract contains several medullated fibres, and a small number of homolateral ascending fibres can be traced from the superior vestibular nuclei into the medial longitudinal fasciculus as far as the trochlear nucleus.

Of the other cranial nerves the oculomotor and trochlear contain only a trace of myelin while the motor root of the trigeminal, abducens, facial and hypoglossal contain slightly more—as do the ventral spinal nerve roots in the cervical region. In addition only the reticulo-spinal fibres contain any myelin. These arise both from the pons and medulla as scattered fibres converging on the medial longitudinal fasciculus (Fig. 2 A) where they turn caudally with or without decussation. A well-marked group of these fibres is seen in all the foetuses (Fig. 1 B) lying just caudal and parallel to the fasciculi of the abducens

Table 2. *Showing the main fibre systems which have acquired a myelin sheath in foetuses of various ages*

tr. = a very few fibres with a fine sheath. + = many fibres with a myelin sheath extending for at least part of their length.

Nerve or tract	Age in days								
	53	63	66	78	96	111	120	129	140
Ventral roots	—	+	+	+	+	+	+	+	+
III, IV, VI, VII, VIII (vest.), XII	—	+	+	+	+	+	+	+	+
V (motor)	—	+	+	+	+	+	+	+	+
Medial longitudinal fasciculus	—	+	+	+	+	+	+	+	+
Vestibulo-spinal, uncrossed	—	+	+	+	+	+	+	+	+
Vestibulo-mesencephalic, uncrossed	—	+	+	+	+	+	+	+	+
Crossed, vestibular	—	+	+	+	+	+	+	+	+
Medullary reticulo-spinal	—	+	+	+	+	+	+	+	+
Pontine reticulo-spinal	—	+	+	+	+	+	+	+	+
Ventral column of spinal cord	—	+	+	+	+	+	+	+	+
Dorsal roots	—	—	+	+	+	+	+	+	+
V sensory mesencephalic and descending	—	—	+	+	+	+	+	+	+
IX, X, XI	—	—	+	+	+	+	+	+	+
Dorsal spino-cerebellar	—	—	+	+	+	+	+	+	+
Uncinate fasciculus	—	—	+	+	+	+	+	+	+
Cerebellar vermis	—	—	+	+	+	+	+	+	+
Lateral column, spinal cord	—	—	+	+	+	+	+	+	+
VIII descending and cochlear	—	—	—	+	+	+	+	+	+
Crossed vestibulo-spinal	—	—	—	+	+	+	+	+	+
Crossed vestibulo-mesencephalic	—	—	—	+	+	+	+	+	+
Isthmic reticulo-spinal	—	—	—	+	+	+	+	+	+
Solitary tract	—	—	—	+	+	+	+	+	+
Ventral spino-cerebellar	—	—	—	+	+	+	+	+	+
Brachium conjunctivum	—	—	—	+	+	+	+	+	+
Myelin in flocculus	—	—	—	+	+	+	+	+	+
Trapezoid body	—	—	—	+	+	+	+	+	+
Lateral lemniscus	—	—	—	+	+	+	+	+	+
Striae acousticae	—	—	—	+	+	+	+	+	+
Rubro-spinal	—	—	—	+	+	+	+	+	+
Mesencephalic reticular at level of inferior colliculus	—	—	—	+	+	+	+	+	+
Myelin in inferior colliculus	—	—	—	+	+	+	+	+	+
Posterior commissure	—	—	—	tr.	+	+	+	+	+
Posterior column of spinal cord	—	—	—	+	+	+	+	+	+
Olfactory tract	—	—	—	—	+	+	+	+	+
Optic nerve and tract	—	—	—	—	+	+	+	+	+
Myelin in lateral cerebellar lobes	—	—	—	—	+	+	+	+	+
Brachium pontis	—	—	—	—	+	+	+	+	+
Commissure of the lateral lemniscus	—	—	—	—	+	+	+	+	+
Peduncle superior olive	—	—	—	—	+	+	+	+	+
Intercollicular commissure	—	—	—	—	+	+	+	+	+
Spino-tectal	—	—	—	—	+	+	+	+	+
Tecto-spinal	—	—	—	—	+	+	+	+	+
Central tegmental fasciculus	—	—	—	—	+	+	+	+	+
Mesencephalic reticular at level of superior colliculus	—	—	—	—	+	+	+	+	+
Stratum profundum of superior colliculus	—	—	—	—	—	+	+	+	+
Supramamillary decussation	—	—	—	—	+	+	+	+	+
Habenulo-peduncular	—	—	—	—	tr.	+	+	+	+
Stria habenularis	—	—	—	—	+	+	+	+	+
Stria terminalis	—	—	—	—	+	+	+	+	+
Fimbria	—	—	—	—	+	+	+	+	+
Optic radiation	—	—	—	—	tr.	+	+	+	+
Olfacto-septal fibres	—	—	—	—	+	+	+	+	+

Table 2 (*continued*)

Nerve or tract	Age in days								
	53	63	66	78	96	111	120	129	140
Fasciculus and ansa lenticularis	—	—	—	—	+	+	+	+	+
Fasciculus thalamicus	—	—	—	—	tr.?	+	+	+	+
Dorsal and ventral supraoptic decussation	—	—	—	—	+	+	+	+	+
Medial lemniscus	—	—	—	—	+	+	+	+	+
Fibres in substantia nigra	—	—	—	—	tr.	+	+	+	+
Efferents from subthalamic nucleus	—	—	—	—	+	+	+	+	+
External capsule	—	—	—	—	tr.	+	+	+	+
Sigmoid and coronal gyri	—	—	—	—	tr.	+	+	+	+
Olivo-cerebellar	—	—	—	—	+	+	+	+	+
Ventral and dorsal external arcuate	—	—	—	—	+	+	+	+	+
Brachium of inferior colliculus	—	—	—	—	—	+	+	+	+
Brachium of superior colliculus	—	—	—	—	—	+	+	+	+
Myelin in stratum superficiale of superior colliculus	—	—	—	—	—	tr.	+	+	+
Fibres in central grey of mesencephalon	—	—	—	—	—	+	+	+	+
Mamillary peduncle	—	—	—	—	—	+	+	+	+
Anterior column of fornix	—	—	—	—	—	tr.	+	+	+
Mamillo-thalamic tract	—	—	—	—	—	+	+	+	+
Efferents from anterior thalamic nucleus	—	—	—	—	—	+	+	+	+
Efferents from ventral thalamic nucleus	—	—	—	—	—	+	+	+	+
Pyramid to spinal cord	—	—	—	—	—	—	+	+	+
Anterior commissure:									
anterior limb	—	—	—	—	—	—	+	+	+
posterior limb	—	—	—	—	—	—	—	tr.	+
Pyriform cortex	—	—	—	—	—	—	—	tr.	+
Corpus callosum	—	—	—	—	—	—	—	tr.	+

nerve. Together these pontine and medullary reticulo-spinal fibres run caudally just ventral to the medial longitudinal fasciculus forming an ill-defined group which comes to lie dorso-medial to the homolateral vestibulo-spinal tract in the ventral funiculus of the spinal cord. Here it is joined by a few fibres from the lateral reticular formation of the medulla which course ventrally through the lateral funiculus of the spinal cord. In foetuses of 78 days (Fig. 1 A) and older these are obscured by a greater number of fibres which enter the lateral funiculus of the spinal cord from the lower medulla and, lying with the rubro-spinal tract, constitute the lateral reticulo-spinal tract. In the 63-day-old foetus a few fibres enter the ventral funiculus of the spinal cord from the ventral reticular formation of the medulla; whether these represent the ventral reticulo-spinal tract or not was not determined.

66-*day foetus*

In this foetus the dorsal spinal roots in the cervical region contain a little myelin, there is some in all parts of the sensory root of the trigeminal nerve, especially the mesencephalic component which can be traced to the level of the inferior colliculus, and in the glosso-pharyngeal, vagus and accessory nerves.

The lateral funiculus of the cervical and upper thoracic spinal cord contains a few medullated fibres of the dorsal spino-cerebellar tract whose fibres pass to the vermis of the cerebellum (Fig. 2 A). A few fibres of the uncinate fasci-

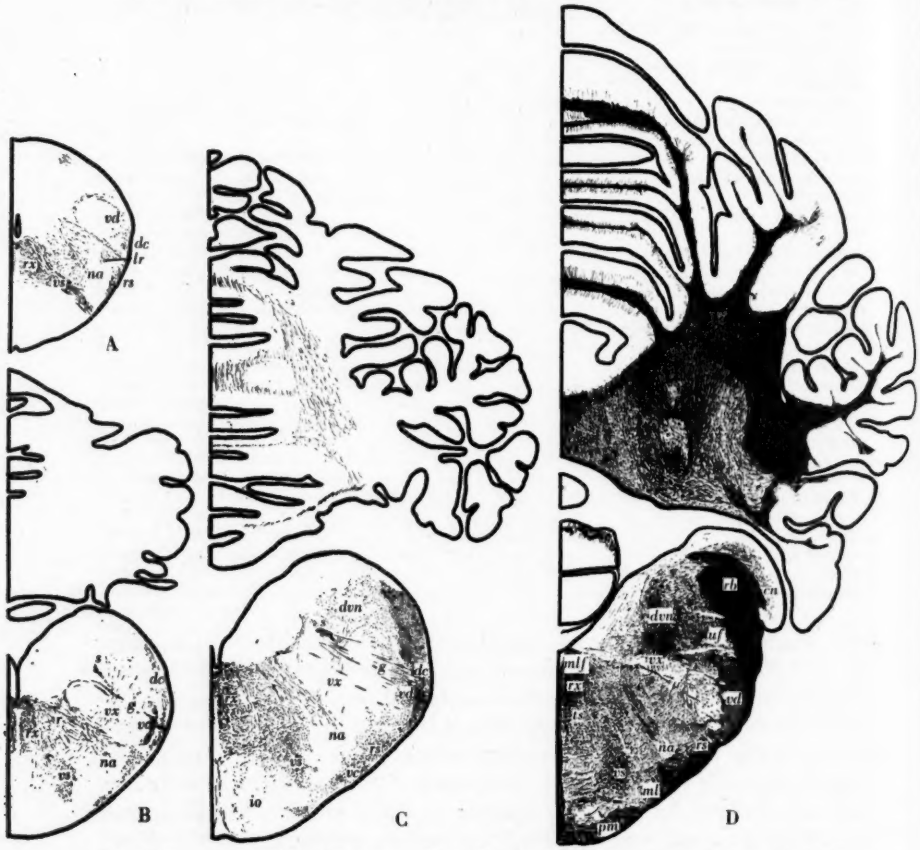

Fig. 1. Drawings of Weigert-Pal preparations of the medulla and cerebellum of three sheep foetuses. A and B, 78-day foetus, ×7·5; C, 96-day foetus, ×7·5; D, 140-day foetus, ×5.

culus arch ventrally from the region of the fastigial nucleus to the vestibular nuclei which also send fibres into the roof nuclei of the cerebellum. The medial longitudinal fasciculus is slightly medullated, especially in its pontine and medullary extent.

78-day foetus

In younger foetuses medullation is confined to the peripheral nerves and central connexions of the vestibular apparatus and the reticulo-spinal pathways. In the 78-day foetus there is a moderate amount of myelin in the

tegmentum of the midbrain (Fig. 5 A). A few fibres of the rubro-spinal tract are medullated and can be traced to the cervical spinal cord. In addition, reticulo-spinal fibres arise from the isthmic region and decussate among a very few fibres of the brachium conjunctivum which cannot be traced forwards to the red nucleus, though the medial longitudinal fasciculus is medullated to the level of the posterior commissure in which are one or two fibres with a myelin sheath.

Fig. 2. Drawings of sections through the vestibular nerve in sheep foetuses. Stain Weigert-Pal. A, 66-day foetus, ×6; B, 78-day foetus, ×7·5; C, 96-day foetus, ×7·5.

All the connexions of the vestibular apparatus are more heavily medullated (Fig. 2 B) and include the cerebellar and descending roots. A few crossed vestibulo-spinal fibres are found arising from the nucleus of the latter (Fig. 1 B), and some crossed vestibulo-mesencephalic fibres from the medial nucleus have a myelin sheath. Both cochlear nuclei contain a few medullated fibres, and these can be traced in the striae acousticae and trapezoid body to a finely medullated lateral lemniscus which reaches the inferior colliculus.

The solitary tract (Fig. 1 B) contains some myelin, and the number of reticulo-spinal fibres from pons and medulla is greatly increased (Figs. 1 B and 2 B).

In the cerebellum the flocculus contains some medullated fibres, and the ventral spino-cerebellar tract can be traced over the brachium conjunctivum into the cerebellum (Fig. 4 A).

The lateral funiculus of the spinal cord contains much more myelin on account of the presence of the rubro-spinal, both spino-cerebellar and the lateral reticulo-spinal tracts, and while a few fibres can be traced in the dorsal fasciculi to the gracile and cuneate nuclei, no internal arcuate fibres are present in the medulla and no collaterals from the dorsal funiculi entering the spinal cord show a medullary sheath. The cervical spinal cord contains more myelin than the thoracic or lumbo-sacral regions. The two foetuses of 78 days show no significant difference in their degree of medullation.

84-*day foetus*

All the funiculi of the spinal cord contain a uniform scattering of medullated fibres without evidence of any localized deficiency. The dorsal funiculi contain the lowest concentration of medullated fibres, and the degree of myelination is uniform throughout the length of the cord with the exception that a few medullated collaterals can be seen entering the dorsal horns from the dorsal funiculi in the upper cervical region.

96-*day foetus*

In this foetus there is a marked increase in the amount of myelin and medullated fibres are present in the forebrain.

Spinal cord. There is a considerable increase in the number of medullated fibres in all funiculi especially the dorsal, and it seems certain that the spino-thalamic tracts are medullated by this stage though they were never seen as separate entities. Many collaterals enter the dorsal horns from the dorsal funiculi in all regions of the spinal cord.

Hindbrain. The main advance in this region consists in the appearance of the medial lemniscus (Figs. 1 C, 2 C, 4 B) which can be traced to the level of the trochlear nucleus. In front of this only a few scattered fibres can be found, and it is doubtful whether the lemniscus reaches the thalamus. In addition, the ventral and dorsal external arcuate and olivo-cerebellar fibres are medullated and longitudinal fascicles in the pontine and medullary reticular formation are much more obvious. The medial longitudinal bundle can be traced to the level of the posterior commissure, where its fibres fan out into the reticular substance above the substantia nigra.

The superior cerebellar peduncle (Fig. 4 B) is well medullated and reaches the red nucleus but cannot be traced for any great distance farther. The descending limb of the brachium conjunctivum (Fig. 4 B), which arises in the decussation, receives fibres from the ipsilateral tegmentum apparently

originating dorsal to the substantia nigra, and can be traced caudally to the pons where a few medullated fibres of the middle cerebellar peduncle are present. The lateral lobes of the cerebellum contain very little myelin.

Midbrain. Fibres of the tecto-spinal and spino-tectal tracts (Fig. 4 B) are medullated, and in the isthmus the central tegmental fasciculus is beginning to be medullated. Its fibres appear to originate mainly from the principal sensory trigeminal nucleus of the same side but could not be traced to the thalamus.

The commissures of the lateral lemniscus and inferior colliculus are medullated.

Lateral to the substantia nigra there is a scattered group of fibres (Fig. 5 B) which seems to be formed partly from the upward continuation of the medial lemniscus and partly from the stratum profundum of the superior colliculus. These can be traced forwards into the lateral part of the fasciculus thalamicus and also give rise to Meynert's commissure. This appearance is in accord with the findings of Magoun & Ranson (1942).

Forebrain. The olfactory and optic tracts contain a considerable number of finely medullated fibres. Optic fibres can be traced to the lateral geniculate body and pretectal nucleus. From the former a few medullated fibres pass into the posterior part of the internal capsule but are so scanty that they cannot be followed to the cortex. Both ventral and dorsal supraoptic commissures contain medullated fibres.

The lateral olfactory tract is medullated over its whole extent, but fibres are not found in the pyriform cortex. From the medial olfactory area a few olfacto-septal fibres pass to the nuclei of the septum pellucidum, and a fine scattering of medullated fibres can be traced to this region in the fimbria but no myelin is present in the anterior column of the fornix.

The habenular and terminal striae both contain a little myelin, and a very few medullated fibres are discernible in the habenulo-peduncular tract.

A small number of fibres with fine medullary sheaths arises from the dorsal part of the frontal cortex. These are almost confined to the sigmoid gyrus but a few are present in the coronal gyrus; they enter the internal capsule and pass caudally as far as the putamen of the lentiform nucleus. In the latter there are a few scattered fibres, and from the globus pallidus a finely medullated bundle forming the fasciculus and ansa lenticularis passes to the subthalamic region. In the subthalamic nucleus there are several myelinated fibres which can be traced to the H_2 Field of Forel.

111-*day foetus*

This stage is not associated with such a great increase in the number of medullated tracts, but rather there is an increase in the degree of medullation of those already seen.

The brachia pontis and all parts of the cerebellar hemispheres now contain a considerable quantity of myelin (Fig. 3 A). In the midbrain (Fig. 5 C) the

brachia of both colliculi are medullated and a few scattered fibres are found in the stratum superficiale of the superior colliculus, with many fibres entering the central grey matter and sweeping ventrally through it towards the oculomotor nucleus and dorsal tegmental decussation. The habenulo-peduncular tract can be followed to the interpeduncular nucleus (Figs. 4 C, 5 C).

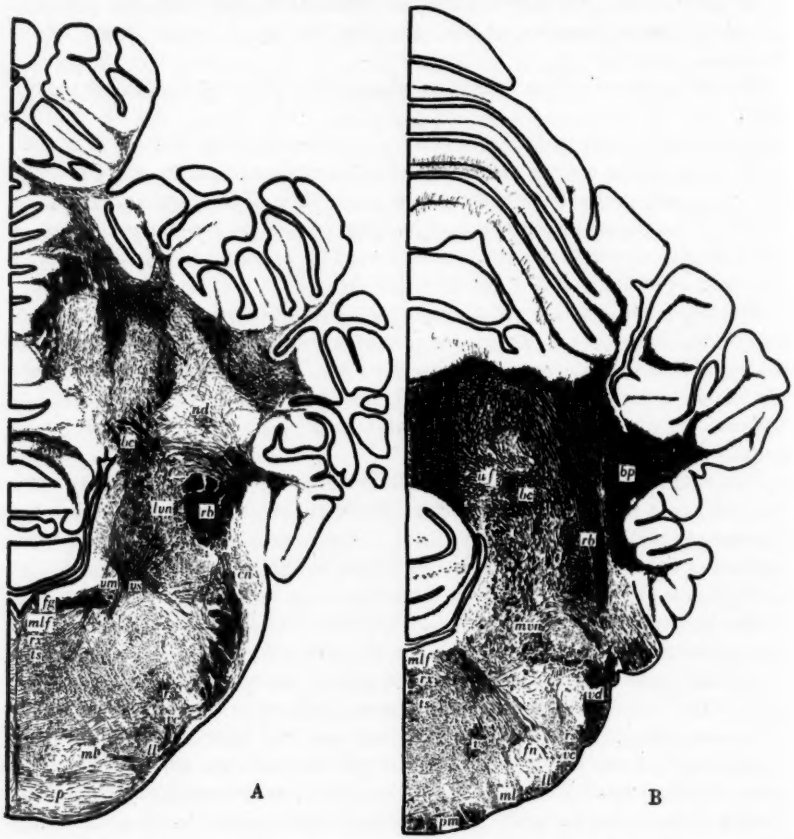

Fig. 3. Drawings of sections through the vestibular nerve in sheep foetuses. Stain Weigert-Pal. A, 111-day foetus, ×6; B, 140-day foetus, ×5.

The mamillary peduncle, the fornix and the mamillo-thalamic tract can all be traced to the mamillary body though the fornix is very poorly medullated.

Fibres of the brachia conjunctiva can now be traced upwards through the red nucleus into the subthalamic region, and both medial lemniscus and spinothalamic fasciculi extend to the thalamus.

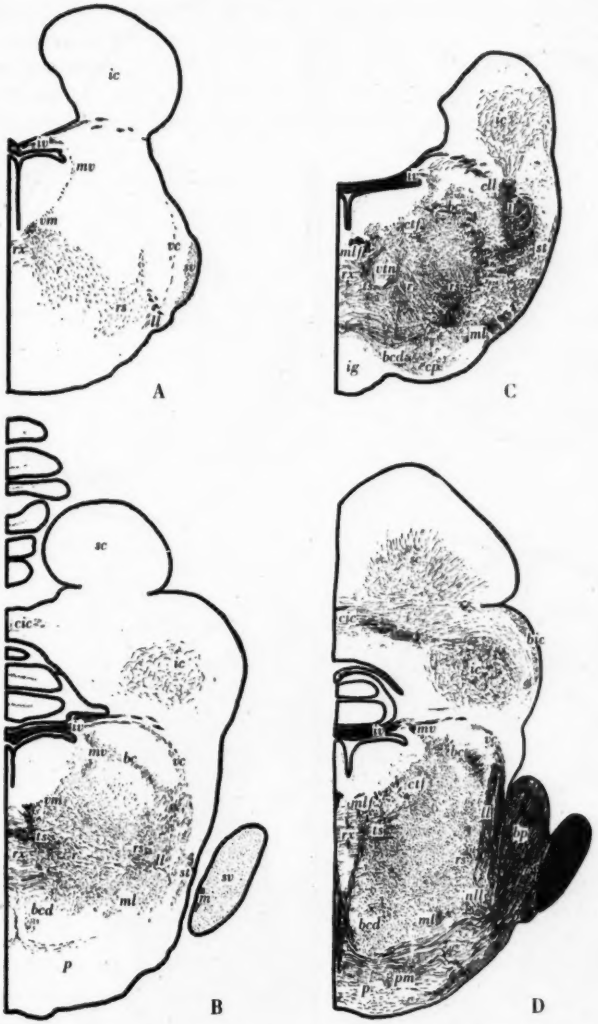

Fig. 4. Drawings of sections through the decussation of the trochlear nerve in sheep foetuses. Stain Weigert-Pal. A, 78-day foetus, ×7·5; B, 96-day foetus, ×7·5; C, 111-day foetus, ×6; D, 140-day foetus, ×5.

Fig. 5. Drawings of sections through the oculomotor nucleus in sheep foetuses. A, 78-day foetus, ×7·5; B, 96-day foetus, ×7·5; C, 111-day foetus, ×6; D, 140-day foetus, ×5.

Most of the thalamic nuclei, with the exception of the pulvinar and dorsal part of the medial nuclear group, contain a few scattered fibres, the anterior nuclei receiving the mamillo-thalamic fasciculus and giving a well-marked group of efferents into the internal capsule (Fig. 6 C), and while the central tegmental fasciculus (Figs. 4 C, 5 C) can be followed to the nucleus reuniens its cephalic part is poorly medullated, though at 140 days (Fig. 5 D, 7 A) this portion is more heavily medullated than the rest.

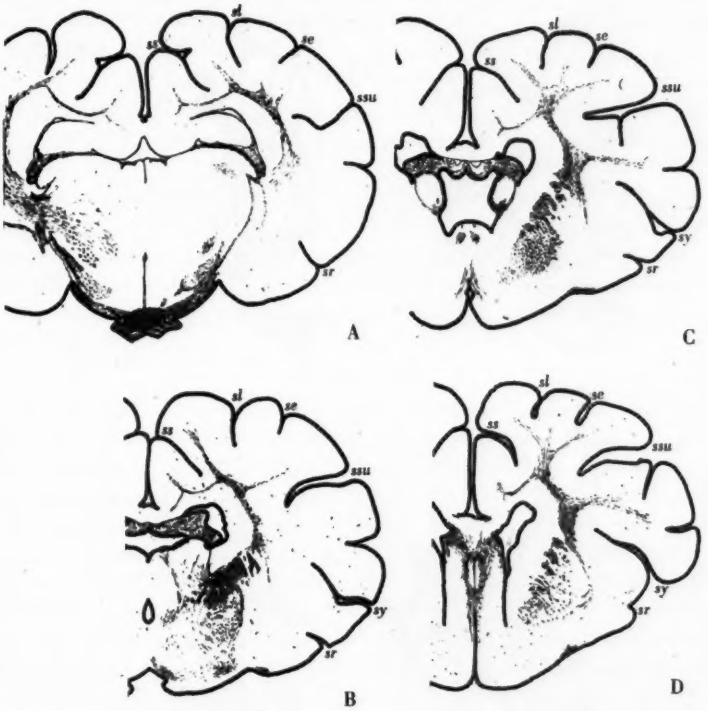

Fig. 6. Drawings of sections through four levels of the forebrain from a sheep foetus of 111 days. Stain Weigert-Pal, ×2.

Efferent fibres are present from the ventral, lateral, anterior, lateral geniculate and medial geniculate nuclei of the thalamus.

The number of medullated cortical connexions is increased though the fibres are not numerous; they are present in the cingulate, coronal, lateral, mid-suprasylvian, posterior suprasylvian, anterior and posterior ectosylvian gyri (Fig. 6).

Efferent cortical fibres can be traced through the corpus striatum into the cerebral peduncle. The great majority of these contain no myelin caudal to

the subthalamic nucleus, but a few (Figs. 5 C, 6 C), whose nature is not determined, pass caudally to the level of the isthmus and appear to end in the ventral part of the tegmentum of the midbrain. There is no myelin in any of the pontine pyramidal fasciculi.

There are no medullated associational fibres in the cortex, and the corpus callosum is devoid of myelin (Fig. 6).

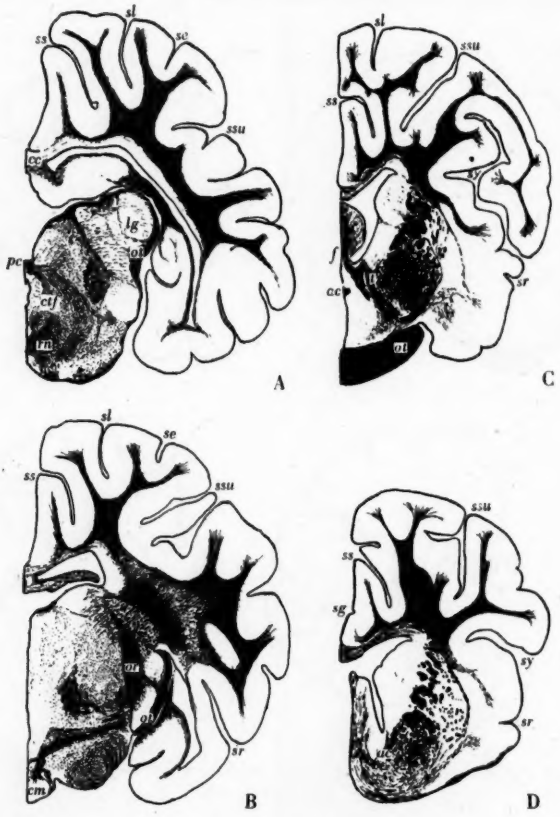

Fig. 7. Drawings of sections through four levels of the forebrain from a sheep foetus of 140 days. Stain Weigert-Pal, ×2.

120-*day foetus*

The central nervous system of this foetus shows no obvious increase in the number of medullated tracts. More myelin is present in the cortex, and a few fibres are found in all regions except the pyriform lobe, the orbital gyri and the extremity of the occipital pole.

Scantily medullated pyramidal fibres can be traced through the pons and a few extend as far caudally as the cervical spinal cord.

All thalamic peduncles contain more myelin and intrathalamic fascicles are more obvious than in the previous foetus, but there is still little myelin in the dorsal part of the medial nuclear group and pulvinar. A few short associational fibres are present in the cortex, but the corpus callosum is devoid of myelin.

The anterior limb of the anterior commissure contains medullated fibres, and though no commissural fibres with a myelin sheath are present in the posterior limb a few derived from the fascicles of the internal capsule run in its lateral part.

130- *and* 140-*day foetuses*

In both of these foetuses all the thalamic connexions are medullated (Fig. 7), and in addition there are medullated fibres in the posterior limb of the anterior commissure (Fig. 7 C) and in the corpus callosum. All parts of the cortex contain several medullated fibres though these are still not numerous in the orbital gyri, the pyriform lobe or the occipital pole; in all regions there are fibres showing the beaded appearance characteristic of early medullation.

The pyramidal tract is much more strongly medullated in the 140- than in the 130-day foetus and can be traced into the upper cervical spinal cord (Figs. 1–3).

DISCUSSION

The present observations on a single series of sheep foetuses cannot be considered exhaustive as there may be considerable variation in the degree of medullation at a given age, not only between different breeds of sheep but also in the same breed. Such a variation is present in the literature dealing with the development of myelin in the human foetus and infant. Table 3 shows the range of variation in the results given in two papers dealing with the first appearance of myelin in man (Lucas Keene & Hewer, 1931; Langworthy, 1933). Some of these variations are very marked involving tracts at all levels of the nervous system, and, though it is not quite clear from Langworthy's (1933) paper how he arrived at a table giving the medullation at ten ages after describing five different ages, it is obvious from his text that his table refers to the first appearance of myelin.

Though the above variations may be the result of differences in material, technical procedures or the level at which some of the tracts are studied, they are too great to allow any conclusions to be drawn about medullation in one foetus and functional development in another.

Despite these differences in man and the fact that the sheep foetuses used in this study have been collected in a random fashion over a period of years, each shows a distinct increase in the degree of medullation over the previous member of the series. Also the two sheep foetuses (268 and 340), 78 days old, show an exactly similar degree of medullation though born in different years. Thus this limited series of observations can be taken to give only a general

survey of the development of stainable myelin in the sheep, and an attempt may be made to correlate this with the development of behaviour only because the material for this paper is part of that used by Barcroft & Barron in their physiological experiments.

Table 3. *Comparing the results obtained by two authors on the development of myelin in man*

Fibre system	Age of medullation in weeks: Lucas Keene & Hewer (1931)	Age of medullation in weeks: Langworthy (1933)	Difference in weeks	Difference as % of smaller observation
Olivo-cerebellar	22	4*	22	100
Ventral spino-cerebellar	14	28	14	100
Tractus cuneatus	14	28	14	100
Tecto-spinal	24–26	4*	18	70
Fornix	32*	8*	24	50
Posterior spinal roots and cranial nerves	14	20	6	43
Dorsal spino-cerebellar	16	20	4	25
Cortico-spinal	36	4*	8	22
Cortico-pontine	Birth	8*	8	20
Habenulo-peduncular	23–24	28	4	16
Medial lemniscus	24	28	4	16
Ventral roots	13–14	16	2	16
Pallido-subthalamic	26–28	32	4	14
Rubro-spinal	36	32	4	11
Mamillo-thalamic	12*	8*	4	7
V (sensory) and VIII (cochlear)	22–24	24	0	0
Optic tract	36	36	0	0

* = weeks after birth.

In general, the order of medullation of the various elements in the nervous system of the sheep agrees with those found in other mammals (Tilney & Casamajor, 1924; Langworthy, 1928 *a*, *b*, 1930, 1933; Lucas Keene & Hewer, 1931, 1933), but the degree of medullation is much more advanced at birth. This precocity of foetal medullation has disclosed the fact that not only do some nerve fibres, such as the ventral spinal roots, become medullated long after there is physiological evidence of their ability to function (Barcroft & Barron, 1942), but also that the optic tract begins to be medullated at least 50 days prior to the exposure of the eye to light.

Unfortunately, it is impossible from the physiological findings to determine exactly which tracts in the nervous system begin to function at a given time, but it has been possible to associate particular regions of the brain with certain phases of development by transecting the brain and spinal cord of older foetuses at various levels and comparing the activity which results with that seen during development (Barcroft & Barron, 1942).

Their results, in conjunction with Table 2, show clearly that activity precedes medullation by a considerable period in all levels of the brain. The onset of inhibition described by Barcroft & Barron (1942) corresponds in time with the first appearance of stainable myelin in the spinal cord, medulla and pons. But by their experiments these authors have demonstrated that when the

midbrain is severed from the pons at this developmental stage, the pontine and medullary segments of the brain demonstrate no evidence of inhibition though they alone contain myelin. Thus the development of myelin is not responsible for the inhibition, the operation merely causing the foetus to revert to a less advanced stage in its functional development without showing any evidence that the presence of myelin in regions caudal to the level of section has altered their functional capabilities. Even when the spinal cord is severed in the cervical region at a time when it contains a moderate number of medullated fibres (70 days), the activity of the trunk and limbs caudal to the section reverts to that found in normal foetuses 36–40 days old when no myelin is present.

There is therefore no evidence that the onset of medullation produces any marked change in the physiological response of the foetus, and the appearance of sustained movements (Barcroft & Barron, 1942) precedes the development of myelin. In contrast to Langworthy's (1926) findings but in agreement with those of Windle (1929), decerebrate rigidity appears in development at a time when the midbrain contains practically no myelin and the rubro-spinal apparatus is quite devoid of it.

These facts are in favour of the view that function precedes medullation and is independent of it for a considerable time. On the other hand, the optic nerve in the sheep begins to be medullated while the foetus has still 50 days to remain *in utero*. Here medullation can be conditioned neither by the onset of normal function in this nervous pathway nor by association with the ability to open the eyes (Langworthy, 1933), for the eyelids are still tightly fused 15 days later and only begin to separate 24 days after medullation in the optic nerve has begun. It may be that the development of myelin in the optic system is initiated by factors quite different from those found elsewhere in the nervous system, but it is clear that the transmission of impulses resulting from light reaching the retina is not a factor in the sheep or in man where the optic nerve also begins to medullate before birth (Lucas Keene & Hewer, 1931; Langworthy, 1933).

The conclusion seems warranted that medullation can occur in the absence of normal function and, though this does not help to clarify the mechanism, that the developing nervous system prepares itself for the demands which are to be put upon it at birth. It is obvious that a sheep is born with a nervous mechanism more highly developed than that of a newborn rabbit, yet each has as little knowledge of the world outside as the other. This inherent development in the absence of function has been demonstrated by Harrison (1904), who has shown that development of functional capabilities in the amphibian nervous system is not retarded by immersion in chloretone which removes from the larva the ability to mould its nervous system by its activity. Similarly, Goodman (1932) states that placing newborn rabbits in the dark for 6 months does not impair medullation of the optic nerve and, though Held (1928) believes that exposure to light hastens this process, it is clear that the absence of function does not prevent or decrease medullation in the optic system.

The connexion between thickness of the myelin sheath, diameter of axon and speed of conduction is well known in the adult nervous system, but the part played by the myelin is not understood. The present study produces evidence that in some cases nerve-fibre systems may function before they develop a medullary sheath, while others become medullated before they are called upon to function. Medullation forms one of the last phases in maturation of the nerve fibre, and it seems likely that tracts once medullated are capable of functioning, though it does not follow that where there is no myelin there is no function. This lack of direct correlation between medullation and function agrees with Angulo y Gonzalez (1929) and Windle (1929) but disagrees with Tilney & Casamajor (1924). The reason for this may be that in studying the early postnatal development of animals such as kittens the process is so rapid that the delay which may exist between function and medullation is eclipsed, but in the paper by Tilney & Casamajor the prenatal behavioural activity is not studied.

SUMMARY

1. The order of medullation is described in many of the tracts in the nervous system of the foetal sheep.

2. Medullation starts at the 63rd day of gestation with the vestibular apparatus, the lower motor neurones and the reticulo-spinal tracts. By 78 days the midbrain connexions have begun to acquire medullary sheaths, and the first myelin appears in the forebrain at 96 days.

3. A comparison of the time of medullation with the functional development described by Barcroft & Barron shows that function in most regions of the brain is established 3–4 weeks before medullation begins. In contrast the optic nerve begins to be medullated 50 days before full term. The relation between the onset of medullation and function is not clear, but it is certain that normal function can occur in the absence of myelin and, in the optic tract at least, medullation begins in the absence of normal function.

4. The possibility is discussed that medullation is an inherent developmental characteristic which is independent of function but may be accelerated by it.

I am indebted to Sir Joseph Barcroft who supplied the material for this study and the figures necessary for computing the age of the two youngest foetuses, which were obtained through the kindness of Dr D. V. Davies, of the Anatomy School, Cambridge.

KEY TO FIGURES

ac	anterior commissure	*cic*	commissure of inferior colliculus
bc	brachium conjunctivum	*cll*	commissure of lateral lemniscus
bcd	descending limb of brachium conjunctivum	*cm*	mamillary body
		cn	cochlear nucleus
bic	brachium of inferior colliculus	*cp*	cerebral peduncle
bp	brachium pontis	*ctf*	central tegmental fasciculus
c	cerebellar root of vestibular nerve	*dc*	dorsal spino-cerebellar tract
cc	corpus callosum	*dvn*	descending vestibular nucleus

f	fornix
fg	facial genu
fn	facial nucleus
g	glossopharyngeal nerve
hp	habenulo-peduncular tract
ic	inferior colliculus
ig	interpeduncular ganglion
io	inferior olive
iv	trochlear nerve
lg	lateral geniculate
ll	lateral lemniscus
lr	lateral reticulo-spinal tract
lvn	lateral vestibular nucleus
m	motor root of trigeminal nerve
ml	medial lemniscus
mlf	medial longitudinal fasciculus
mp	mamillary peduncle
mv	mesencephalic root of trigeminal nerve
mvn	medial vestibular nucleus
na	nucleus ambiguus
nd	nucleus dentatus
nll	nucleus of lateral lemniscus
o	oculomotor nerve or nucleus
or	optic radiation
ot	optic tract
p	pons
pc	posterior commissure
pm	pyramid
r	reticular fibres
rb	restiform body
rn	red nucleus
rs	rubro-spinal tract
rx	medial reticulo-spinal tract and decussation
s	solitary tract
sc	superior colliculus
se	sulcus ectolateralis
sg	sulcus cinguli
sl	sulcus lateralis
so	superior olive
sr	sulcus rhinalis
ss	sulcus splenialis
ssu	sulcus suprasylvianus
st	spino-tectal tract
sv	sensory root of trigeminal nerve
svn	superior vestibular nucleus
sy	sulcus sylvianus
t	stria terminalis
tb	trapezoid body
ts	tecto-spinal tract
uf	uncinate fasciculus
v	vestibular nerve
vc	ventral spino-cerebellar tract
vd	descending trigeminal root
vi	abducens nerve
vii	facial nerve
vm	vestibulo-mesencephalic tract
vs	uncrossed vestibulo-spinal tract
vtn	ventral tegmental nucleus
vx	crossed vestibulo-spinal
x	crossing vestibular fibres

REFERENCES

Angulo y Gonzalez, A. W. (1929). *J. comp. Neurol.* **48**, 459.

Barcroft, J. & Barron, D. H. (1936). *J. Physiol.* **88**, 56.

Barcroft, J. & Barron, D. H. (1937). *J. Physiol.* **91**, 329.

Barcroft, J. & Barron, D. H. (1939). *J. comp. Neurol.* **70**, 477.

Barcroft, J. & Barron, D. H. (1942). *J. comp. Neurol.* **77**, 431.

Goodman, L. (1932). *Amer. J. Physiol.* **100**, 46.

Harrison, R. G. (1904). *Amer. J. Anat.* **3**, 197.

Held (1928). Quoted from Mann, I. C., *The Development of the Human Eye.* Cambridge.

Langworthy, O. R. (1926). *Contr. Embryol. Carneg. Instn*, **17**, 125.

Langworthy, O. R. (1928*a*). *Contr. Embryol. Carneg. Instn*, **20**, 127.

Langworthy, O. R. (1928*b*). *J. comp. Neurol.* **46**, 201.

Langworthy, O. R. (1930). *Contr. Embryol. Carneg. Instn*, **21**, 37.

Langworthy, O. R. (1933). *Contr. Embryol. Carneg. Instn*, **24**, 1.

Lucas Keene, M. F. & Hewer, E. E. (1931). *J. Anat., Lond.*, **66**, 1.

Lucas Keene, M. F. & Hewer, E. E. (1933). *J. Anat., Lond.*, **67**, 522.

Magoun, H. W. & Ranson, M. (1942). *J. comp. Neurol.* **76**, 435.

Tilney, F. & Casamajor, L. (1924). *Arch. Neurol. Psychiat.* **12**, 1.

Windle, W. F. (1929). *J. comp. Neurol.* **48**, 227.

CYCLICAL CHANGES IN THE ISLETS OF LANGERHANS IN THE RAT PANCREAS

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INTRODUCTION

Certain discoveries have opened up the possibility of studying, under controllable conditions, the morphological changes associated with the functioning, growth, degeneration and regeneration of the islet tissue of the pancreas. The discoveries referred to are: (*a*) that by Shaw Dunn, Sheehan & McLetchie (1943) of the development of glycosuria and the production of a necrosis of the cells of the islets of Langerhans after the parenteral administration of Alloxan to rats and rabbits; (*b*) the demonstration by Hughes, Ware & Young (1944) that the necrotic action was practically confined to the β cells, and was almost immediate in onset, and their suggestion that the action of the drug was a specific one on the β cells; (*c*) the proof of this suggestion by Goldner & Gömöri (1944), Ridout, Ham & Wrenshall (1944), and Goldner (1945).

As a result of a comparative study of groups of rats treated for long periods with small doses of Alloxan and groups of normal animals, Hughes & Hughes (1944) suggested the theory that a cycle of formation, growth, maturation, and destruction of the islets is a normal feature of the pancreas of the adult rat.

The present paper presents further evidence in support of this hypothesis.

MATERIAL

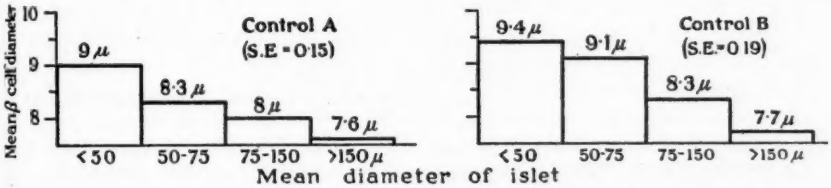
The material consisted of approximately fifty rats of an inbred black and white variety, and ten albino rabbits, also of an inbred strain. So far as possible litter-mates were used in each group of experiments. The experiments were carried out by Prof. F. G. Young of University College, London, Dr L. L. Ware of Lincoln College, Oxford, and Dr C. N. N. Vass of St Thomas's Hospital Medical School, London, and it is to their kindness that the writer owes the opportunity of carrying out the histological investigations. All material for histological examination was fixed in Bouin's or Helly's fixative, embedded in paraffin, cut at 4 μ and stained by Gömöri's technique (1941).

The rats were divided into groups of five animals—litter-mates—of which one, the control, received saline only. The others received doses of Alloxan varying from 300 mg./kg. of bodyweight to 1 mg./kg. In some groups treatment consisted of a single dose of Alloxan, in others the drug was administered daily for periods varying from 2 weeks to 3 months. In the latter cases the daily dose never exceeded 125 mg./kg. It has been found that the minimum single diabetogenic dose is 200 mg./kg.

There are considerable species differences both in the normal structure and in the reaction of the pancreas to treatment. In this paper, accordingly, only the results of the experiments with rats will be reported.

RESULTS

In the pancreas of the normal healthy adult rat there is a considerable variation in the size of the islets of Langerhans. A few islets are less than 50 μ in mean diameter and consist of about 10–20 β cells with, at most, 2–3 α cells. The cells of such islets are readily distinguished from centro-acinar and duct cells, with which they might be confused, by the presence within them of the specific granules. Most of the islets in the pancreas are found to have a mean diameter of 50–150 μ. A few giant islets with a mean diameter of considerably more than 150 μ are usually present. The α cells form a thin layer, seldom exceeding one cell in thickness, on the surface of all the islets except the smallest. On close examination of the islets it is obvious that the size of the β cells diminishes in proportion to increase in the mean diameter of the islet. Furthermore, in the large islets some cells appear to be necrotic. The nuclei are pyknotic and the cell bodies shrunken and distorted. There are always some such degenerating cells present which cannot be recognized as β cells owing to the fact that they do not contain the specific granules (Pl. 1, figs. 1, 2).



Courtesy of 'The British Journal of Experimental Pathology' and Messrs H. K. Lewis and Co.

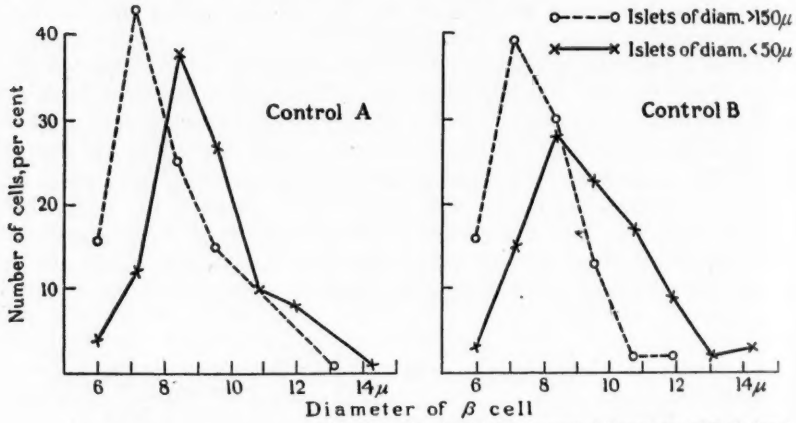
Text-fig. 1. Diagram to show the inverse relationship between the mean diameter of β cells and the mean diameter of the islet in the normal rat. S.E. = standard error of difference between mean for islets below 50 μ and islets above 150 μ in diameter.

In order to provide a statistical basis for further investigation a number of measurements were made with the eyepiece micrometer in a series of five normal animals. For this purpose the islets were classified arbitrarily as follows:

(*a*) Islets of mean diameter 50 μ or less;
(*b*) Islets of mean diameter 50–75 μ;
(*c*) Islets of mean diameter 75–150 μ;
(*d*) Islets of mean diameter 150 μ and above.

In each animal 400 typical β cells were measured, 100 from each category of islet. Cells devoid of the specific granules were ignored. Each cell was measured on both its long and short axis and the mean diameter calculated. The mean β-cell diameter was then calculated for each category of islet. The results in all the control animals were agreeably consistent. The relationship between mean β-cell diameter and size of islet in two of these animals is shown graphically in Text-fig. 1. It is clear that the mean β-cell diameter decreases steadily with increase in the size of the islet.

A distribution curve showing the incidence of cells of varying size in these same pancreases (Text-fig. 2) demonstrates their symmetrical distribution about a peak which, in the case of the large islets, lies at about 7 μ and in the case of the small islets at about 8·5 μ. The largest cells in the small islets are always larger than the largest cells in the large islets.



Courtesy of 'The British Journal of Experimental Pathology' and Messrs H. K. Lewis and Co.

Text-fig. 2. β cell distribution curve, showing the percentage incidence of β cells of different diameters in islets below 50μ and above 150μ mean diameter in the normal rat pancreas.

A series of animals was then treated with a single intraperitoneal diabetogenic dose of Alloxan (300 mg./kg. body-weight). The animals were divided into three groups as follows:

GROUP A. Five animals:

	Alloxan treated (4)	Saline treated (1)
Animal killed	5 min. later	—
	10 ,,	—
	15 ,,	—
	30 ,,	Control killed

GROUP B. Five animals:

	Alloxan treated (4)	Saline treated (1)
Animal killed	15 min. later	—
	30 ,,	—
	45 ,,	—
	60 ,,	Control killed

GROUP C. Five animals:

	Alloxan treated (4)	Saline treated (1)
Animal killed	2 hr. later	—
	6 ,,	—
	12 ,,	—
	24 ,,	Control killed

In these animals changes could be detected in the larger islets within 5 min. of the administration of the drug. Most of the β cells in such islets show varying

degrees of nuclear pyknosis, cytoplasmic degranulation and irregularity of cell outline. The narrow pericapillary spaces become widened.

As the time interval between the administration of the drug and removal of the tissue is increased from a few minutes to 24 hr. the changes become more marked and more extensive, involving the smaller islets to an ever-increasing degree. In certain animals, however, some small islets show little or no sign of damage at any time, even several days after the drug has been given (Pl. 1, fig. 3*a*, *b*). It seemed likely, therefore, that by careful adjustment of the dose of Alloxan it might be possible to produce a selective necrosis of the larger islets only so that the role of the smaller islets in the regeneration of the islet tissue might be studied.

Fifteen female rats were divided into groups of three. The animals in Group 1 served as controls and received a daily dose of saline only. Group 2 animals were treated daily with 125 mg. Alloxan/kg., administered subcutaneously. Group 3 animals received 25 mg./kg., Group 4 animals 5 mg./kg. and Group 5 animals 1 mg./kg. One animal from each group was killed after 14 days, a second after 1 month and the remaining animals after 2 months.

After 14 days of treatment with 125 mg. of Alloxan all the large islets showed degenerative changes. In some the cells were in various stages of degranulation; in others shrunken, distorted and necrotic β cells occur. A few large islets consist entirely of peculiar agranular cells. The small islets are all unchanged. If treatment is continued with this dose for a month large islets become few in number. Some show degenerative changes in the β cells but the majority appear healthy. These latter, however, contain β cells which are larger than normal. The small islets are undamaged (Pl. 1, fig. 4). After 2 months of treatment with 125 mg./kg. there are no large islets and very few small islets in the pancreas. Even the latter show evidence of damage to β cells (e.g. degranulation and pyknosis).

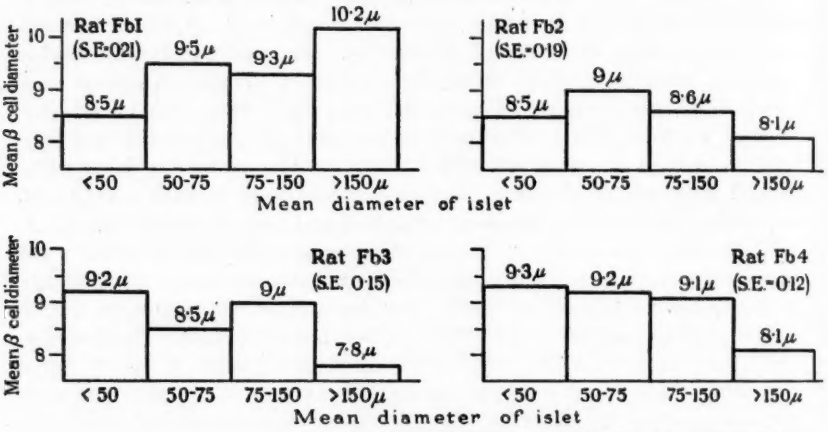
With 25 mg. doses no changes can be detected until after 2 months of treatment. Then the changes are slight, consisting in peculiarities in the distribution of the β granules within the cytoplasm, or of cloudy swelling of the β cells. With 5 mg. and 1 mg. doses no obvious changes can be detected at any time.

The plotting in graph form of the β cell distribution for these pancreases shows, however, that significant changes occur in the islets of almost all the treated animals (Text-fig. 3). There is a complete reversal in the distribution of the cells in the case of the animals receiving the higher doses of Alloxan. The larger islets now contain the larger cells, and show two frequency peaks at 8·5 and 11·8 μ. The largest cells in the large islets now exceed in size the largest cells in the small islets (Text-fig. 4). There is little change in the small islets.

In the case of animals treated with smaller doses of Alloxan the disturbances become less striking as the dose is reduced, although even after a dose of 1 mgm. daily there is a tendency for the average size of the β cells to be greater than the normal in all islets.

It would seem reasonable to conclude from these experiments that the smaller β cells which are so striking a feature in the larger islets are more sensitive to the action of the toxic agent and are destroyed by it. As they are destroyed they are replaced by new, large, β cells, which are presumably young cells (derived from a source to be discussed later).

These new β cells almost certainly produce insulin since none of the animals in the chronic experiments developed glycosuria. It has been shown above, however, that in the normal animal the relative number of large cells increases with diminution in the size of the islet until, in the smallest islets, all the β cells are of the large type. It appears likely that in the normal untreated animal small islets are young islets and large islets, which so consistently show



Courtesy of 'The British Journal of Experimental Pathology' and Messrs H. K. Lewis and Co.

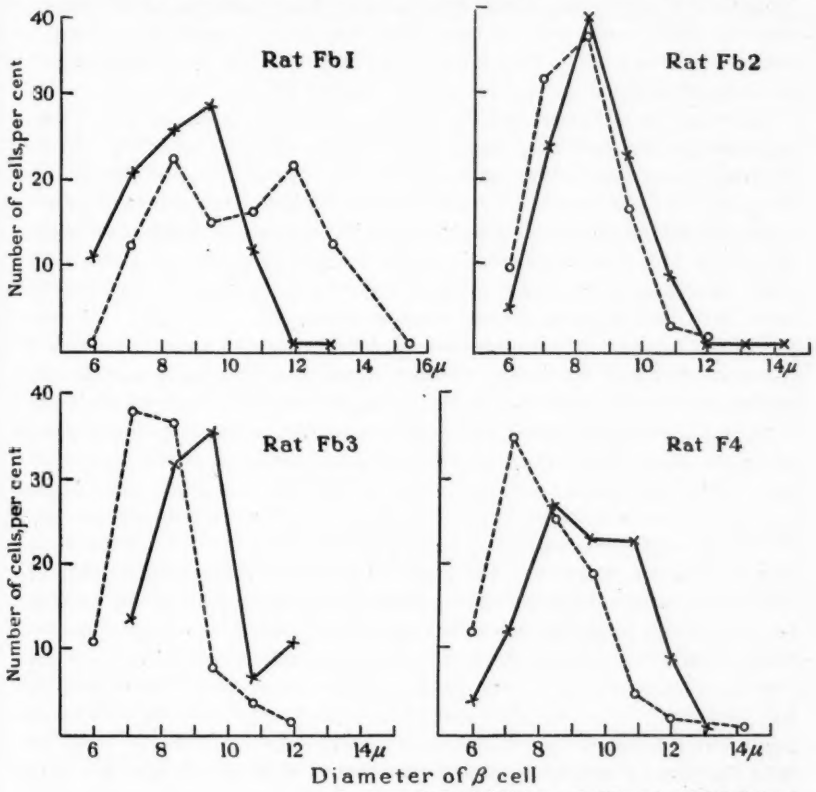
Text-fig. 3. Showing the disturbance of the normal relationship between cell size and islet size after the administration of Alloxan. Rat Fb1 received 125 mg. Alloxan/kg. daily for 1 month; rat Fb2 received 25 mg. Alloxan/kg. daily for 1 month; rat Fb3 received 5 mg. Alloxan/kg. daily for 1 month; rat Fb4 received 1 mg. Alloxan/kg. daily for 1 month.

evidence of cellular death among the smaller β cells, old islets. This involves the assumption that the small, large-celled islets of the normal animal enlarge into the large, small-celled islets; but this view cannot be accepted without evidence that active growth does, in fact, occur in small islets.

It has been shown by Hughes *et al.* (1944), and by many other workers, that mitosis among the β cells during the stage of regeneration of islet tissue following Alloxan-induced necrosis is very rare indeed. Furthermore, mitotic figures are found only very occasionally among the β cells of normal animals or of animals in which a partial pancreatectomy has been recently performed, and in which, therefore, some hypertrophy of the islet tissue may be anticipated. This problem of the source or sources of new islet cells, and especially of new

β cells has attracted the attention of many workers and numerous suggestions have been made. Three of these suggestions have been supported by evidence based upon much experimental work. They may be summarized as follows:

(*a*) That increase in the amount of islet tissue is the outcome of mitotic proliferation of the islet cells. This view has been adopted by some recent workers on experimental diabetes, either alone (Richardson & Young, 1938)



Courtesy of 'The British Journal of Experimental Pathology' and Messrs H. K. Lewis and Co.

Text-fig. 4. β cell distribution curves, showing the alteration in the 'spread' of the β cells in the large islets after treatment with Alloxan. (Dosage as indicated in Text-fig. 3.)

or in conjunction with one or both of the following hypotheses (Woerner, 1938).

(*b*) That the islets are budded off from the duct system of the gland. Weichselbaum (1908) appears to have been the first to carry out partial ablation experiments in the attempt to confirm this theory. He removed a portion of the pancreas in dogs and guinea-pigs and noted that mitoses

occurred chiefly in the duct cells (but also in the acinar and islet cells). He concluded that islets and acinar tissue arose independently from the duct epithelium. Bensley (1915) came to a similar conclusion as a result of his study of the guinea-pig pancreas.

(*c*) That acinar tissue can be converted into islet tissue. This hypothesis seems to have originated with Laguesse (1908) as a part of his theory of 'balance'. He held that acinar tissue and islet tissue were convertible the one into the other, each mass of tissue functioning alternately as a source of external secretion in the form of acini and as a source of internal secretion in the form of islets.

The direction of change and the relative amounts of acinar and islet tissue depend upon the functional requirements of the animal at the time. In this form the theory won little support, but many workers since the time of Laguesse have observed an apparent transformation of acinar into islet tissue under conditions which might be supposed to increase the demand for insulin within the organism (Herxheimer, 1906; Otami, 1927; Ohmori, 1930; Falin, 1932; Woerner, 1938). There seems to be no real evidence in favour of the belief that the reverse transformation can occur.

The examination of the pancreas of Alloxan-treated animals appears to offer strong support for the theory of acino-insular transformation as the chief source of new islet cells. The study of serial sections of such glands makes evident certain structural features which appear to have been overlooked hitherto. In the pancreas of normal rats some of the small but none of the large islets show points of acino-insular transformation (Pl. 2, fig. 5*a*), but such points are seldom found to be multiple on the surface of any one islet. Where the acinar tissue abuts upon the islet tissue the distinction between the two is usually a sharp one. The acinar cells are very much larger than the islet cells, contain numerous eosinophilic zymogen granules at the terminal pole and much basophilic material at the basal pole. The islet as a whole is surrounded by a delicate connective tissue capsule and the α and β cells contain their specific granules. At the points of acino-insular transformation the connective tissue capsule seems to disappear and the acinar cells at this point lose the basophilic material and become filled towards the basal pole with granules exhibiting the staining properties of the β cell granules. These acinar cells are often binuclear, and in such cases one nucleus appears to migrate into the basal portion of the cell which is then separated off by the development of a cell membrane so that this basal element appears to be budded off as a large β cell into the islet (Pl. 2, fig. 5*a*). In those cases where some islet tissue has been destroyed by Alloxan and regeneration is presumably occurring points of acino-insular transformation become much more numerous and in serial section can be found at several places on the surface of every islet (Pl. 2, fig. 5*b*). It has been noted that the α cells in the rat islet lie peripherally. In cases where regeneration is proceeding actively the new β cells budded off from the surrounding acini displace the α cells centrally (Pl. 2, fig. 6).

In the course of the present investigations it has not proved possible to gain any clear evidence regarding the regeneration of α cells owing to the fact that these cells are undamaged by moderate doses of Alloxan. It seems likely, however, that acinar cells can give rise to β or α cells as need may be. In connexion with this acino-insular transformation it is perhaps significant that Duff & Starr (1944) found in the hooded rat a great increase in the mitotic activity of the *acinar* cells during the first 24 hr. after the injection of Alloxan.

DISCUSSION

The evidence on which the hypothesis of a cycle of growth and decay in the islets of the rat pancreas rests may be summarized as follows:

There are invariably signs of cellular degeneration and death in the large islets of the normal pancreas. Occasionally large islets undergoing hyalinization are encountered (Pl. 2, fig. 7).

The large islets of the pancreas contain small β cells, the small islets large β cells.

Small islets almost invariably show some evidence of acino-insular transformation. The acinar cells appear to be converted into large β cells. By means of graded doses of Alloxan a differential necrosis of the small β cells of the large islets can be produced. This stimulates a more extensive acino-insular transformation and the replacement of the dead cells by large β cells, which are laid down peripherally in the islets. The α cells are thus displaced centrally as a mass so that the rat islet becomes very unusual in appearance. But even in the normal pancreas some few α cells are found occasionally in a central position. The shell of α cells never forms a complete investment for the mass of β cells and is always interrupted at the point of acino-insular transformation.

The theory draws additional support from the fact that it suffices to explain certain anomalous findings in experiments on the effects of ligation of the pancreatic duct. Falin (1932) noted that the sugar tolerance in the domestic fowl was increased after ligation of the pancreatic duct. Herxheimer (1926) ligated the duct in hens and found, 36 days after operation, that the blood-sugar fell in starvation to the convulsive level and that the islet tissue had undergone hypertrophy. Bierry & Kollmann (1927) observed an hypertrophy of the endocrine tissue after ligation of the duct of the pancreas. Fahr (1914) found that partial pancreatectomy in a dog did not produce glycosuria unless the dog was kept alive for a year, even though only a very small portion of the pancreas was left behind. Demel & Kramer (1928) found that in the dog ligation of the duct led to a postoperative hypoglycaemia, followed 11 months later by a rise in the blood-sugar. Burkhardt (1936) had the opportunity of studying two cases of carcinoma of the head of the pancreas in man with obstruction of the pancreatic duct. In both cases apparent transformation of terminal alveoli into islet cells was noted, and in one case it was possible to show that a period of 6 months elapsed between the onset of symptoms of obstruction of the duct and the occurrence of glycosuria. Retterer (1927)

demonstrated that after ligation of the pancreatic duct in a dog, much, but by no means all, of the acinar tissue was destroyed by the time the animal was killed 7 months later. The surviving acinar tissue had, however, become modified in structure so as to form a series of anastomosing cords of cells all well granulated.

It is suggested that after ligation of the pancreatic duct much of the acinar tissue survives because it is already destined to become transformed into islet tissue. The stimulus of ligation, by preventing the cells functioning as exocrine cells may indeed in some cases speed up the transformation so that the animal acquires more islet tissue than is required for its immediate needs and suffers from hypoglycaemia. In most cases, however, the acinar tissue remains in a resting state until with the gradual atrophy of the already mature islets, a demand for new islet cells arises. The acinar tissue then undergoes the acino-insular transformation. Only when this supply of acinar tissue is exhausted does the animal manifest signs of insulin insufficiency. This requires a period of from 6 months to a year in the case of men and larger mammals such as the dog.

CONCLUSION

Obviously much remains to be done before these hypotheses of the transformation of acinar tissue into islet tissue, and of the slow growth and decay of the islets themselves can be accepted without reservation. Especially is it necessary to clear up much that is obscure in connexion with the relationship between the α cells, the acini and β cells. It is interesting to note, however, that a somewhat similar growth, death and replacement of cells has been postulated, Salmon & Zwemer (1941), Baxter (1946), in that other gland which is so intimately connected with carbohydrate metabolism—the suprarenal cortex.

The reconstruction of the connective tissue and especially of the vascular supply involved in the development of islets from acinar tissue would seem to offer no insuperable difficulties. Such radical changes are by no means uncommon in the genital tract of the female.

SUMMARY

1. A statistical study has been made of the mean diameters of the β cells in islets of varying size in the pancreas of the normal rat.

2. It is shown that with increase in the mean diameter of the islet there is a decrease in the mean diameter of the β cells.

3. By the use of graded doses of Alloxan it is possible to produce a differential necrosis of the small β cells of the large islets, and to study the details of regeneration of the β cells.

4. It is suggested that the small diameter β cells are senescent, that they are being replaced by a transformation of acinar cells into large diameter β cells, and that this is indicative of the occurrence in the normal pancreas of a cycle of growth and decay of the islets.

5. An attempt is made to explain, on the basis of this hypothesis, some of the conflicting results of experimental interference with the pancreas.

It is a pleasure to acknowledge my indebtedness to Miss J. V. Maddox who has been of great assistance to me in the production of many of the sections and to Mr J. King who prepared the photographs. Prof. Young, Dr Ware and Dr Vass will publish the results of their physiological investigations elsewhere.

REFERENCES

BAXTER, J. S. (1946). The growth cycle of the cells of the adrenal cortex in the adult rat. *J. Anat., Lond.*, **80**, 139–146.

BENSLEY, R. R. (1915). The structure and relationships of the islets of Langerhans-criteria of histological control in experiments on the pancreas. *Harvey Lect.* **10**, 250–289.

BIERRY, H. & KOLLMANN, M. (1927). La théorie du balancement et le pancréas des téléostéans. *C.R. Soc. Biol., Paris*, **97**, 1382–1383.

BURCKHARDT, L. (1936). Inselneubildung im Pankreas bei Stenose des Ausführungsganges durch Pankreaskopfcarcinom. *Virchows Arch.* **296**, 655–665.

DEMEL, H. & KRAMER, E. (1928). Lässt sich die innere Sekretion der Bauchspeicheldrüse auf operativem Wege beeinflussen? *Mitt. Grenzgeb. Med. Chir.* **41**, 114–120.

DUFF, G. L. & STARR, H. (1944). Experimental alloxan diabetes in hooded rats. *Proc. Soc. exp. Biol. N.Y.* **57**, 280–282.

DUNN, V. S., SHEEHAN, H. L. & MCLETCHIE, N. G. B. (1943). Necrosis of islets of Langerhans produced experimentally. *Lancet*, **1**, 484–487.

FAHR, TH. (1914). Diabetes-Studien. XIV. *Virchows Arch.* **215**, 247–270.

FALIN, L. (1932). Langerhanssche Inseln und Blutzucker nach Unterbindung der Ausführungsgänge der Bauchspeicheldrüse. *Virchows Arch.* **284**, 713–753.

GOLDNER, M. G. (1945). Alloxan diabetes; its production and mechanism. *Bull. N.Y. Acad. Med.* **21**, 44–55.

GOLDNER, M. G. & GÖMÖRI, G. (1944). Studies on the mechanism of alloxan diabetes. *Endocrinology*, **35**, 241–248.

GÖMÖRI, G. (1941). Observations with differential stains on human islets of Langerhans. *Amer. J. Path.* **17**, 395–406.

HERXHEIMER, G. (1906). Über Pankreascirrhose (bei Diabetes). *Virchows Arch.* **183**, 228–341.

HERXHEIMER, G. (1926). Pankreas-Zellinseln und Insulin nach Unterbindung der Ausführungsgänge der Bauchspeicheldrüse. *Klin. Wschr.* **5**, ii, 2299–2302.

HUGHES, H. & HUGHES, G. E. (1944). Effects of prolonged administration of small doses of alloxan upon islet tissue of the rat pancreas. *Brit. J. exp. Path.* **25**, 126–130.

HUGHES, H., WARE, L. L. & YOUNG, F. G. (1944). Diabetogenic action of alloxan. *Lancet*, **1**, 148–150.

LAGUESSE, E. G. (1908). Sur les rapports des îlots endocrines avec l'arbre excréteur dans le pancréas de l'homme adulte. *C.R. Soc. Biol., Paris*, **65**, 139–141.

OHMORI, M. (1930). Histologische Untersuchung der Langerhansschen Inseln des Pankreas mit Rücksicht auf ihren Sekretionsprozess. *Okayama Igakkai Zasshi*, **42**, 777–787.

OTAMI, S. (1927). Studies on the islands of Langerhans in the human pancreas. I. The relation of the island to the surrounding structures. *Amer. J. Path.* **3**, 1–12.

RETTERER, E. (1927). Evolution des éléments du pancréas après la résection de ses canaux excréteurs. *C.R. Soc. Biol., Paris*, **96**, 22–25.

RICHARDSON, K. C. & YOUNG, F. G. (1938). Histology of diabetes induced in dogs by injection of anterior pituitary extracts. *Lancet*, **1**, 1098–1101.

RIDOUT, J. H., HAM, A. W. & WRENSHALL, G. A. (1944). Correlation of insulin content and histological picture of pancreas at intervals after administration of alloxan. *Science*, **100**, 57–58.

SALMON, T. N. & ZWEMER, R. L. (1941). A study of the life history of cortico-adrenal cells of the rat by means of Trypan-blue injections. *Anat. Rec.* **80**, 421–429.

WEICHSELBAUM, A. (1908). Über die Regeneration der Langerhans'schen Inseln im menschlichen Pankreas. *S.B. Akad. Wiss. Wien*, Abt. III, **117**, 211–225.

WOERNER, C. A. (1938). Studies of the islands of Langerhans after continuous intravenous injection of dextrose. *Anat. Rec.* **71**, 33–57.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Normal rat pancreas ($\times 700$). (*a*) Islet $<75\mu$ in diam.; (*b*) islet $>150\mu$ in diam. The decrease in the mean diameter of the β cells is evident.

Fig. 2. Normal rat pancreas ($\times 400$). Note the peripheral position of the α cells in the large islet. (*a*) Islet $<75\mu$ in mean diam.; (*b*) islet 75–150μ in mean diam.; (*c*) islet $>150\mu$ in mean diam. The large islet shows necrotic β cells (pyknotic nuclei, irregular outline to cells, fragmentation of cells, etc.).

Fig. 3. Pancreas 4 days after a single intraperitoneal dose of 300 mg./kg. of Alloxan ($\times 700$). (*a*) Large islet showing extensive necrosis; (*b*) small islet showing little sign of damage.

Fig. 4. Small islet after one month of daily treatment with 125 mg./kg. of Alloxan ($\times 700$). The β cells all appear healthy and heavily granulated. Two points of acino-insular transformation can be seen.

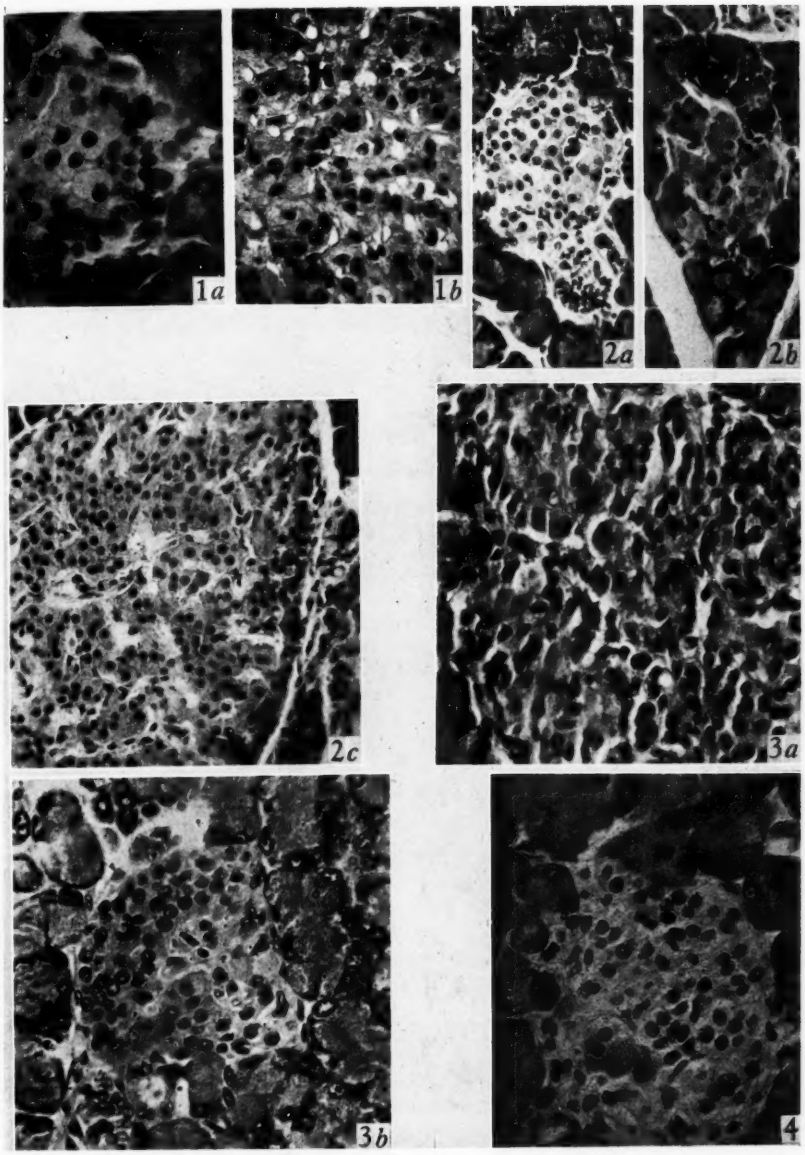
PLATE 2

Fig. 5, *a*. Periphery of medium islet in pancreas of normal rat ($\times 600$). In the centre one large acinar cell appears to be dividing or casting off part of its cytoplasm (containing a second nucleus) into the islet. In the slide it can be seen that the staining properties of the granules of this portion of the cell are changing so as to approximate those of the β granules.

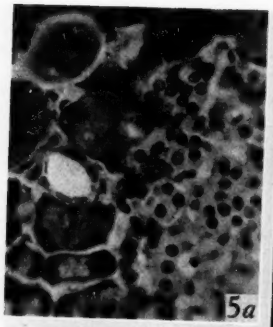
Fig. 5, *b*. Small islet in pancreas of rat treated 4 days before death with 200 mg./kg. of Alloxan. Acino-insular transformation is occurring at the upper left-hand corner of the islet ($\times 600$).

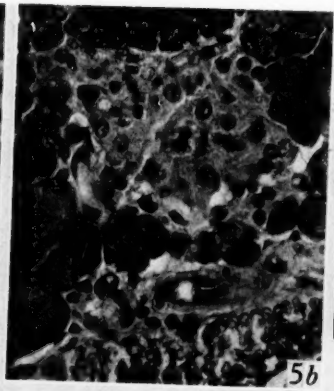
Fig. 6. Part of a medium islet ($\times 750$) showing the darker α cells displaced from the peripheral position by development of β cells from acinar cells (on left and at bottom of photograph).

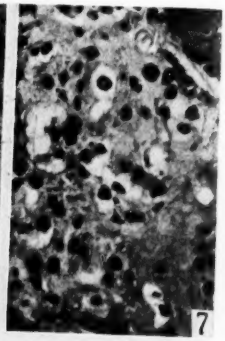
Fig. 7. Portion of large degenerating islet from normal rat pancreas ($\times 600$). Slight hyalinization present.

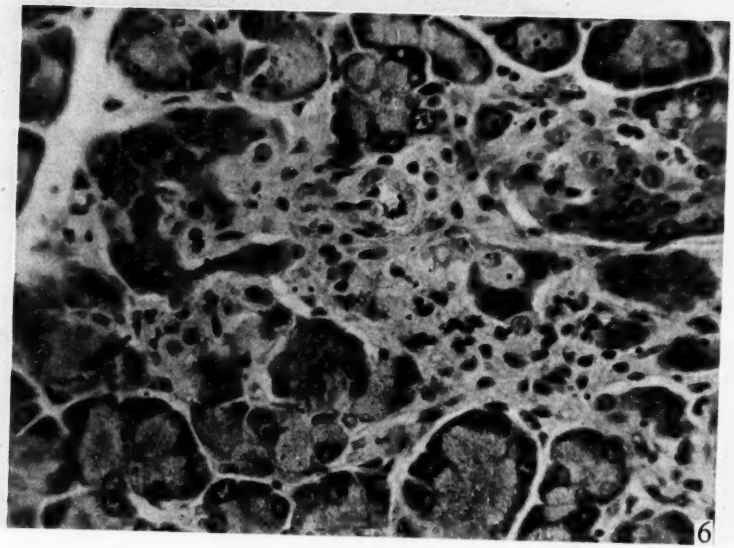


HUGHES—Cyclical changes in islets of Langerhans in rat pancreas









HUGHES—Cyclical changes in islets of Langerhans in rat pancreas

THE DEVELOPMENT OF THE SPECIALIZED CONDUCTING TISSUE OF THE HUMAN HEART

By E. W. WALLS, *Anatomy Department, University College, Cardiff*

INTRODUCTION

Views which have long been held with regard to the morphological nature of the specialized conducting tissue of the mammalian heart have recently been strongly challenged by Davies (1942). He believes that the system in mammals and birds is a neomorphic development and '. . . is not a "remnant" of more extensive tissues of similar structure in lower vertebrate hearts'. In support of this thesis Davies cites as collateral evidence the findings of Shaner (1929) in the developing calf heart. According to that author the atrio-ventricular (A.V.) node appears in the calf embryo at the 9 mm. stage as an excrescence on the part of the A.V. ring that lies behind the dorsal endocardial cushion, at a time when the developing musculatures of the atria and ventricles are in unbroken continuity. As it develops the node grows forwards on to the free edge of the inter-ventricular septum, becoming flask-shaped as it does so, and by the 23 mm. stage the pointed end of the flask has lengthened out into the Y-shaped A.V. bundle and its two limbs. It therefore would seem that the A.V. node and bundle are derivatives but not remnants of the original A.V. ring. The sino-atrial (S.A.) node is not recognizable in the calf embryo till much later, 100 mm., but it too possesses a true individuality, appearing at a definite time and growing and differentiating in a regular manner. It is first seen as a structurally specialized part of the antero-lateral surface of the superior vena cava just above the atrium, but its appearance has been heralded for some time by the gradual accumulation of nerve cells and fibres in the region. As will be seen later Shaner's description corresponds closely to what occurs in human embryos; it differs, however, in some particulars from the account given by Calcagno (1941 *a*, *b*), who states that the A.V. node and bundle arise separately in the bovine embryo and become joined secondarily.

Accounts of the development of the conducting tissue of the human heart are few, accompanying photographic illustrations are fewer still, and only one writer, Sanabria (1936), has described the development of the entire system. But even more than these considerations, what prompted the present study was the desire to establish if possible whether the nodes and bundle appear, grow and develop as do the other organs of the body, or whether they are simply remnants of the junctional tissues found at the S.A. and A.V. rings.

The first attempts to identify the A.V. bundle in the human embryo succeeded only in fairly advanced specimens. Thus Tawara (1906) identified it at 100 mm., Keith & Flack (1906) at 45 mm., Fahr (1907) at 160 mm. and

Monckeberg (1908) at 75 mm. The position of the bundle on the summit of the inter-ventricular septum was explained by Keith & Flack as due to the fact that the upper edge of the septum represents the least disturbed part of the interior of the embryonic heart and the part which during systole is least affected. Histological differentiation of the bundle was described at the 165 mm. stage by Monckeberg, who also noted that in the embryo the limbs of the bundle behave as in the adult, the left branching early and the right remaining unbranched, and that their Purkinje fibres do not appear until quite late.

Amongst the earlier workers in this field Tandler (1912) and Mall (1912) inclined to different opinions with regard to the origin of the human A.V. bundle. According to Mall the bundle is an embryological remnant of the atrial canal of which the posterior musculature, connecting the sinus and the ventricle, never breaks down although showing early in development changes in structure which differentiate it from the rest of the heart muscle. In an embryo of 11 mm. C.R. length Mall was able to identify the bundle which was becoming isolated by the breaking down of the rest of the A.V. ring. He also claimed that in suitable sagittal sections the bundle may be recognized as early as the 8 mm. stage and, indeed, figures it in a specimen measuring only 7 mm.

Tandler, on the other hand, seemed to favour the view that the bundle does not represent the persistence of an ancient A.V. connexion but is a new development, the conclusion which had been reached by Retzer (1908) from his studies of the developing pig. In 10 mm. human embryos in which the atria and ventricles are continuous at the A.V. canal the bundle, according to Tandler, can be recognized as a strip of cells of darker colour on the posterior wall of the canal, and by 19·75 mm. the bundle can be seen on the summit of the inter-ventricular septum and its limbs followed a short way. The problem of the conduction of the impulse from atria to ventricles engaged Tandler's attention, and he pointed out that conduction in hearts without a ventricular septum must be different from that in hearts with one, just as it must differ in the same heart before and after the completion of the septum during development.

Sanabria described histological differentiation in, and a typical reticular arrangement of, the cells of the posterior atrial wall in a 6 mm. human embryo. By the 11 mm. stage he found the muscle of the posterior atrial canal noticeably different from that near it, although the general A.V. muscular continuity was then still unbroken. From its lower end this differentiated muscle gave rise to a line of cells which sank into the dorsal endocardial cushion to reach as far as its ventral free border. So placed, this muscular band, which was clearly the A.V. bundle, capped the upper border of the inter-ventricular septum just where it joined the dorsal cushion. Sanabria did not discuss whether or not he regarded the bundle as a remnant of the original A.V. connexion, but from his description it would appear that while the A.V. node is a part of the original muscular connexion between atrium and ventricle which undergoes exceedingly early differentiation, the bundle, on the other hand, is a new formation derived from this tissue by active proliferation into the dorsal endocardial cushion.

PRESENT WORK

Material used in the present investigation was as follows:

A. Complete serial sections of the following embryos:

No. 162. Cardiff Collection, C.R. length 8 mm.
No. 161. Glasgow Collection, C.R. length 8 mm.
No. H. 33. London Hospital Collection, C.R. length 10 mm.
No. H. 23. London Hospital Collection, C.R. length 13 mm.
No. 129. Cardiff Collection, C.R. length 16·5 mm.
No. 44. Cardiff Collection, C.R. length 18·8 mm.
No. 110. Cardiff Collection, C.R. length 22 mm.
No. 114. Cardiff Collection, C.R. length 25 mm.
No. 7. Cardiff Collection, C.R. length 88 mm.

B. Complete serial sections of the hearts from embryos of 40, 60 and 78 mm. C.R. length.

C. Interrupted serial sections of the hearts from foetuses of 105, 123 and 150 mm. C.R. length.

D. Interrupted serial sections of blocks from still-birth hearts.

All the material was cut at 10 μ except embryo H. 33 which was cut at 8 μ and embryo no. 7 which was embedded in celloidin and sectioned at 100 μ. Embryo 110, the hearts from the 105, 123 and 150 mm. foetuses, and some of the still-birth material were impregnated with silver before cutting. All other sections were stained with haematoxylin and eosin.

To facilitate precise orientation of the specialized conducting tissue wax-plate reconstructions were made of the hearts of the 16·5 and 60 mm. embryos.

The main planes of section of the different hearts examined will be indicated as they are described, but it must be stressed that complete series of true transverse, sagittal or coronal sections of developing hearts cannot be obtained as the curvature of the embryo and of the heart itself cause the plane of section to change as cutting proceeds.

For convenience the usual terms will be used in describing the endocardial cushions, but it is felt, nevertheless, that anterior and posterior as usually employed are unsatisfactory, and that there is some justification for Frazer (1931) reversing the customary usage.

OBSERVATIONS

8 *mm.*

Two embryos of this size, each cut transversely, were studied. In one no specialized tissue could be found in the heart but in the other, no. 161, sections 19.1.9 to 19.2.7, the tissue which will become the definitive A.V. node and bundle can be identified. At this stage there is complete continuity between the atrial and ventricular primitive musculatures, and at one point, on the right side of

the posterior wall of the common atrium at its junction with the ventricle, a localized proliferation of tissue can be seen budding off. As it grows forward it passes under the dorsal endocardial cushion, still not fused with the ventral, to reach the summit of the inter-ventricular septum which has by now succeeded in reaching the dorsal cushion in this position. The appearance presented is unquestionably that of an actively growing structure (Pl. 2, figs. 1, 2). Even at this early stage it is possible to recognize differences between the node and the bundle. The node is less compact than the bundle, which, in addition, stands out by reason of the very great density with which the nuclei of its cells stain. In cross-section the bundle presents a somewhat oval appearance and, as observed by Mall in sagittal sections of an embryo of similar size, is already becoming separated off although the spaces which surround it are as yet small. The bundle can be followed for a short distance down the left side of the septum.

In Sanabria's description of the posterior musculature of the A.V. canal in the 6 mm. human embryo it is stated that the fibres there show less advanced fibrillary differentiation and are paler than their neighbours. These features were not noted by Mall in 8 mm. embryos, nor were they observed in embryos of that size in the present study.

It should be clearly understood that in order to be able to identify the A.V. bundle at such an early stage as 8 mm. experience of its appearance in older embryos is essential, and although the present description necessarily proceeds from the younger to the older stages the investigation was carried out in the reverse way.

10 *mm.*

The plane of section of this embryo is transverse, but although the upper parts of the atria are cut in this plane the greater part of the heart has been cut in vertical section. By now, although the developing musculatures of the atria and ventricles are still largely in unbroken continuity, in places signs of commencing breakdown are evident. Pl. 2, figs. 3 and 4, show one such place, where on the left side only a slender strand of muscle connects the common atrium with the ventricle. These sections illustrate also that, as pointed out by Mall, the breakdown in muscular continuity begins about the time of appearance of the lateral endocardial cushions. On the posterior wall of the A.V. canal but towards its right side, there is a clearly defined, almost circumscribed, body of cells, which when traced serially can be recognized as the A.V. node from which the bundle is now very obviously arising (Pl. 2, fig. 3), and passing upwards into the base of the dorsal endocardial cushion. The node is in cellular continuity with the wall of the atrium but fibrous tissue, the future trigonum fibrosum dextrum, is filling in the A.V. sulcus and separating it from the ventricle. Tissue spaces around the developing bundle are now conspicuous, and these, together with its large deeply staining nuclei, make identification certain. There can be no question that whereas the A.V. node represents a part of the original A.V. canal destined to become structurally specialized *the bundle*

is a new formation which arises from that primitive nodal tissue by a process of active growth.

The point where the nodal tissue joins the musculature of the right atrium is exactly the place at which the lower end of the right venous valve is attached (Pl. 2, fig. 3), so that in effect the A.V. node and right venous valve are at this stage continuous with one another. This brings to mind the connexion described by Tudor Jones (1932) between the S.A. and A.V. nodes in a 24 mm. human embryo by a tissue strand within the right venous valve.

When the bundle is traced farther it is found to run down the left side of the inter-ventricular septum for a short distance, but as yet no right limb is recognizable (Pl. 2, fig. 4). This agrees with Mall's statement that in the early stages of its development the bundle is related more to the left ventricle than to the right. Neither in the ordinary myocardial cells nor in those of the differentiating conducting tissue are cell outlines easily discernible. Some cells in the left limb of the bundle show vacuole formation (Pl. 3, fig. 5), a most interesting feature suggesting as it does extreme precocity in the histogenesis of Purkinje fibres. Incidentally, when Purkinje fibres do appear they do so first in the upper part of this limb.

The sinu-atrial node. At the S.A. junction at the 10 mm. stage there is a round clump of cells more closely packed together than those of the neighbouring tissue (Pl. 3, fig. 6). The compactness of the cell group and its position rather than any specific histological character of its individual cells suggest it as the anlage of the future pacemaker, although their nuclei do show a slightly stronger chromatic reaction than those of adjacent cells. Traced caudally this tissue can be found in the upper parts of the bases of both venous valves.

In older embryos there develop in close association with the S.A. node numerous ganglion cells from which nerve fibres pass into it, and in addition the artery which traverses the node, and which forms such a conspicuous feature in the adult, serves as a means of identification. But the special histological characters of the nodal fibres are not recognizable till birth. It is, maybe, as well to emphasize that these do not at any time constitute anything very striking, and in fact several investigators deny any structural specificity even of the adult node, but they are, nevertheless, real enough, e.g. smaller breadth compared with that of ordinary atrial fibres and, in the writer's opinion, less marked cross-striation.

Sanabria (1936) also described a localized thickening of the heart wall at the junction of the superior vena cava and the right atrium in an embryo of 11 mm., composed of fibres noticeably more compressed and paler than those of the contiguous muscle.

Examination of older embryos confirms that these aggregations of cells noticed at 10 and 11 mm. do in fact represent the S.A. node. It might be thought that, while the specialized conducting tissue can be recognized as early as 8 mm., its development is then in such a primitive state as to preclude any possibility of functional activity; however, there are solid grounds for the belief that thus

early (8 mm. = approx. 38 days) the system is functioning as in the adult. This point will be discussed later under 'Foetal electrocardiography'.

13 *mm.*

Main plane of section transverse. The A.V. bundle is now very easily identified. Not only has it developed into a denser and more 'rounded-off' structure, but the tissue spaces around it have enlarged so that in places it seems quite isolated (Pl. 4, figs. 10, 12). If these figures are compared with figs. 24–28 of Mall's paper it will be appreciated that in the embryo here described, H. 23, the bundle has by 13 mm. reached a degree of differentiation equal to if not in advance of that reached by Mall's 21 mm. specimen. The bundle now bears its definitive relationship to the septal cusp of the tricuspid valve. The left limb of the bundle is now of considerable size, and fibres from it can be followed into the developing trabecular tendons, sometimes called false tendons, which bridge the ventricular cavity (Pl. 4, fig. 10). For the first time it is now possible to identify the right limb of the bundle; it is represented by one or two strands of cells which descend from the main bundle along the right side of the upper part of the inter-ventricular septum (Pl. 4, fig. 11).

The sinu-atrial node. Within the basal attachment of the fused venous valves below the opening of the sinus venosus there is a kidney-shaped mass of cells which forms the entire thickness of the heart wall between epicardium and endocardium. This is the future posterior horn of the S.A. node (Pl. 4, fig. 9). Traced upwards it just fails to become continuous with a similar cell clump situated within the upper part of the base of the right venous valve. In this specimen, therefore, the S.A. node is developing in two parts, an upper and a lower, which represent respectively the anterior and posterior horns of the adult node. There is still no artery traversing the node, but its cells stand out by reason of their greater affinity for eosin. The caudal end of the node extends along the right venous valve for a short distance as the valve passes to be attached to the dorsal endocardial cushion; farther down the heart the A.V. node is continuous with the attachment of the right valve to the dorsal cushion, but no direct connexion between the two nodes at this or any other stage has been found.

16·5 *mm.*

Main plane of section transverse. The A.V. node and bundle have now reached a considerable size and in many respects possess the topographical relationships of the fully developed structures. The bundle is very sharply defined (Pl. 1). It describes an arch upwards and slightly forwards from the node to the upper part of the inter-ventricular septum at its junction with the dorsal endocardial cushion. The point of junction is over towards the right side of the cushion, now really the fused endocardial cushions, an important point in the development of the pars membranacea septi and one which is well shown in the figure. The bundle then passes along the upper margin of the septum below the still largely

patent inter-ventricular foramen, a position in which it has been successfully dissected in congenitally malformed hearts by Keith (1909). Differences in the conformation of the two limbs of the bundle are now apparent. In the 16·5 mm. embryo examined the left limb arises as a series of strands which begin to come off the main bundle some time before the right limb which is now more clearly defined and in effect the direct continuation of the bundle. This manner of division of the bundle corresponds to type C of the adult (Walls, 1945) and was the type most frequently met with in the series of embryos studied. Another point of difference between the limbs now is their relation to the endocardium. From its commencement the left limb is immediately subendocardial,whereas the right limb passes downwards for a short distance through the substance of the inter-ventricular septum before reaching its inner surface. According to Matsuda (1936) the course of the right limb in the foetus varies greatly, and he figures three possible courses which it may take, all of which are in part at least within the muscle of the septum. His findings, together with those of Sanabria (1936) and those of the present investigation, explain why it is that, in the adult, dissection of the right limb of the A.V. bundle varies so greatly in its ease of accomplishment. Clearly if the bundle maintains a position largely within the muscular septum then its exposure must necessarily be exceedingly difficult if not impossible.

In the bundle cell outlines are becoming clearer, and some of the fibres of the lower part of the right limb are noticeably larger than those of the adjacent myocardium and contain large sausage-shaped nuclei. Rondolini (1937) stated that the cells of the system can now be recognized by their larger volume and more swollen appearance, but these characters were only noted at 16·5 mm. by the present writer in some of the cells of the right limb of the bundle as mentioned above. There is still no evidence of a connective tissue sheath around the conducting tissue.

The A.V. node has its fibres arranged in a loose open meshwork which is in continuity with the right atrium and with the bundle, the latter providing its only connexion with the ventricle.

In places the muscular continuity between the atria and ventricles has not yet been cut through by the invading fibrous tissue of the A.V. sulcus, and in Pl. 1 the left atrium and left ventricle can be seen still joined by a persisting part of the old A.V. canal. Muscular slips of this kind may well have been what Kent (1893) described as the first and third of his three connexions between the atria and ventricles of the mammalian heart. In most cases, of course, these muscle slips disappear, leaving the A.V. bundle as the sole connexion between atria and ventricles, but there is reason to believe that occasionally they do persist and give rise to phenomena which can be recognized clinically (Wolff, Parkinson & White, 1930; Ohnell, 1946).

The sinu-atrial node. The further development of the S.A. node is witnessed by the appearance of a small nodal artery within the tissue of the base of the right venous valve. Moreover, there can now be seen for the first time in any

part of the heart primitive nerve cells. These occur on the epicardial aspect of the posterior surface of the common atrium, partly about halfway down this surface from where later on they can migrate into the inter-atrial septum and so supply the A.V. node, but mainly in relation to the S.A. junction. These cells do not as yet have clear-cut cell borders, possibly due to delay in fixing the embryo, their cytoplasm forming a bright pink syncytium in which are set nuclei possessing that perfect roundness so characteristic of nerve cells. The source of the intrinsic (vagal, parasympathetic) cardiac nerve cells is still in doubt, opinion being divided between a sympathetic and a vagal origin. For this reason the closest attention was given to this question, but unfortunately without shedding further light upon it. It would seem that for problems of this kind only the experimental method can provide the answer, and such work carried out by Jones (1942) indicates strongly a vagal origin for these cells.

18·8 *mm.*

This embryo was the only member of the series examined which was cut sagittally, and although in the writer's opinion this plane of section is not so generally useful for a study of the cardiac conducting tissue as transverse or coronal, some valuable information was obtained.

In Pl. 3, fig. 7, the S.A. node can be identified within the substance of the crista terminalis at the bottom of the sulcus separating the superior vena cava from the right atrium. It is placed towards the sinus side of the crista, the position which it occupies in the adult (Blair & Davies, 1935). The appearance presented by the sinus, atrium and ventricle lying in series in sagittal section in an embryo of this size is very reminiscent of an adult lowly vertebrate heart, and makes it easy to understand why the S.A. and A.V. nodes should have been accepted by earlier workers as the remnants of primitive junctional tissue. The first to object to this conception was Stiénon (1926), who described the S.A. node as a new growth that is properly located at the junction of the common cardinal vein and the sinus venosus and not as remnant tissue left over from a mass of similar character and more widespread distribution. This description has been confirmed. The position of the A.V. node close to the opening of the coronary sinus is well shown in this embryo, but the bundle is very difficult to find and follow as in no part of its course is it cut through its full thickness. In the sections in which it can be seen it is surrounded by a delicate connective tissue sheath. This investment never really increases during subsequent development, the explanation no doubt of the universal failure to reveal the conducting system by injection in the human heart as in the ox. The cells of the bundle as it passes between the fibrous tissue of the A.V. sulcus and the dorsal cushion are closely packed together and appear somewhat narrower than those of the unspecialized myocardium. On reaching the muscular septum the bundle swells out a little and its cells become broader, but none show any of the characters of Purkinje fibres.

22 *and* 25 *mm.*

New features noted in these transversely cut embryos are these: The right limb of the bundle can now be traced into the moderator band, and nerve cells are definitely present on the posterior surface of the atria at 25 mm. It is interesting to note that the silver impregnation method by which the 22 mm. specimen was prepared failed completely to show any nerve elements in the heart. His, Jr. (1893), stated that nerves arrived at the sinus region about the 12·5 mm. stage, but Mall (1912) was unable to find them in that situation till 21 mm. No doubt there is a fair range of variation in the development of embryos, although possibly the variation exists more in the measurement of their specimens by different observers. Stiénon (1926) reported a 22·4 mm. embryo as the youngest showing ganglion cells in the region of the sinus or atria and asserted that their appearance preceded that of the S.A. node. The same author did describe, however, in embryos of 6·4–15 mm. C.R. length, differences in the character of the muscle of the superior vena cava...greater density, increase in numbers of nuclei, etc....similar to those accepted by later writers as indicating the presence of the nodal anlage.

The A.V. node at 25 mm. (Pl. 3, fig. 8) forms a large mass of loosely arranged muscle fibres in the interstices of which capillary blood vessels are becoming numerous. The nodal fibres are thinner than those of the adjacent myocardium and are without a demonstrable nerve supply.

40 *mm.*

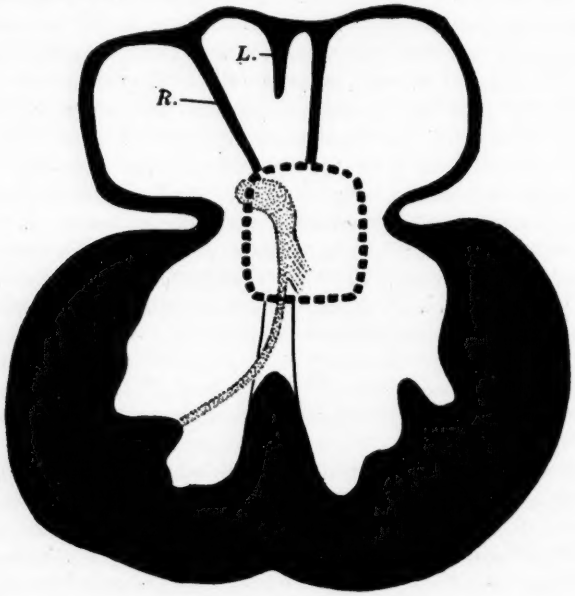
The plane of section is transverse, but much of the ventricles appears to have been cut coronally.

The S.A. node has now established itself in its adult form, i.e. an open horseshoe in front of the opening of the superior vena cava into the right atrium. Although in the 13 mm. embryo examined there was some evidence that the node consisted of two separate parts, it is not possible to agree with Segre (1926) who stated that only after birth do the anterior and posterior horns of the node become joined together. At 40 mm. the part of the node which lies in the crista terminalis and which connects its two horns is definitely present. It is shown in Pl. 5, figs. 13 and 14, together with the nodal artery here cut along its length.

Nor is it possible on physiological grounds to accept Segre's findings. The circumstances of his investigation were these: A patient with mitral stenosis gave an electrocardiogram in which a double auricular wave existed. This suggested the presence of two S.A. nodes, one for the right and one for the left atrium. From his examination of the hearts of two full-time foetuses, one newborn child and one young male adult, all cut at 30 μ, Segre decided that the node did in fact develop in two parts and that the case in question was an instance of the persistence of the node in two parts. Now if he were correct then electrocardiograms of foetal hearts should exhibit the phenomenon displayed by the case of presumed double node. But they do not: human foetal

heart tracings are just like those of the adult which, even allowing for the small size of the heart, would be hardly likely if the S.A. node were in two parts.

Numerous nerve fibres derived from groups of cells in the sinus walls are now entering the node, but at no time are nerve cells to be found within the node. Tudor Jones (1932) described a communication between the caudal end of the S.A. node and the right phrenic nerve in a 24 mm. human embryo: in a few sections of the 40 mm. embryo here described the phrenic nerve is adherent to the surface of the lower part of the node, but no fibres from it can be seen



Text-fig. 1. Diagram to show the position of the A.V. node and bundle in the developing heart. The interrupted line represents the dorsal endocardial cushion. *R* and *L*, right and left venous valves.

entering the node either in this specimen or in any of the others examined. For the most part, as shown in Pl. 5, fig. 13, the nerve lies well clear of the heart wall.

The atrio-ventricular node and bundle. Pl. 5, figs. 15 and 16, show the bundle before and at division as well as many features of general interest in the development of the heart. As the bundle runs forward on the summit of the fleshy inter-ventricular septum it now bears its close and important relation to the mitral and tricuspid valves. It is relatively huge at this stage, being in section fully a third of the thickness of the septum. Its fibres and those of the limbs show well-developed myofibrillae (Pl. 5, fig. 17) but no cross-striations;

as yet they are unaccompanied by nerve fibres. The arrival of nerve fibres in the sinus wall was suspected by Mall to hasten the histological differentiation of the conducting fibres, and the present study confirms this suspicion. The appearance of Purkinje fibres in the limbs of the bundle occurs simultaneously with that of nerve fibres at about 100 mm. The A.V. node has quite a rich nerve supply by the 40 mm. stage, thick leashes of unmyelinated nerve fibres running forwards into it from the atrial septum (Pl. 5, fig. 18).

Since subsequent to 40 mm. the disposition of the specialized conducting tissue remains unaltered a brief recapitulation at this point may prove of value: The A.V. node represents part of the original A.V. canal musculature which becomes structurally differentiated early, and from it the A.V. bundle sprouts forwards into the base of the dorsal endocardial cushion. By continued new growth the bundle reaches the attachment of the inter-ventricular septum to the fused cushions and there divides, the type of division varying, but in most cases being that in which the left limb arises as a series of strands.

105 *mm.*

In vertical section the bundle is now seen to occupy a groove in the upper border of the fleshy septum, just below the pars membranacea septi, immediately beneath the septal attachment of the tricuspid valve (Pl. 6, fig. 19). Both the bundle fibres and those of the myocardium now show cross-striation, but with silver impregnation the bundle fibres are much more lightly coloured and distinguishable with ease. Nerve fibres can be seen running within the bundle and the upper parts of both limbs; they are few in number and seem non-myelinated, although with silver it is always difficult to be dogmatic on this point. In any event they are very fine fibres which show slight beading along their length and end as delicate threads on the surface of the conducting muscle fibres. In the upper part of the left limb, but nowhere else, a few Purkinje fibres have appeared, this being the youngest embryo in which these fibres were observed. Sanabria (1936) described Purkinje fibres in the bundle and upper part of the left limb in an embryo of 60 mm.

123 *mm.*

A notable feature of this embryo is the marked advance in the development of the cardiac ganglia in relation particularly to the sinus part of the atria. At no time before birth are there two cell types in these ganglia as in the adult human heart (Blair & Davies, 1935). The single type which occurs in the foetus corresponds generally with type 1 of these authors, and although the cells are rather darker this feature may well be due to slight differences in preparation.

150 *mm.*

The A.V. node has now a rich blood and nerve supply. Connective tissue fills the spaces between its freely branching striated fibres, the whole structure being somewhat more closely knit than hitherto. Purkinje fibres are now

plentiful in both limbs of the bundle (Pl. 6, figs. 22, 23). Many of these fibres possess all the characters of the large bloated 'typical' Purkinje fibres of the ox or sheep, i.e. they are subendocardial in position, they are on the average about twice the breadth of adjacent ventricular fibres, 11μ compared with 5μ; they exhibit cross-striation only at their periphery, the myofibrillae being limited to the cortex of each fibre; some of the fibres show vacuolation and, while the majority have a single large round or oval centrally placed and (with silver) pale nucleus, a number have two nuclei. In none of the Purkinje fibres could peripheral multiple nucleation as described in the ox by Blair & Davies (1935) be observed. In general the fibres of the right limb seem smaller than those of the left, a feature which has been remarked upon by others also, but this may be due to the difference in direction of the two limbs with the consequent effect upon the planes of section of their constituent fibres. It must be pointed out that although figures have been quoted above of fibre diameters the precautions stated by Ashley (1945) to be necessary for complete accuracy in measurement could not in the nature of the investigation be taken. No Purkinje fibres occur elsewhere than in the subendocardium. Many of the fibres in the limbs of the bundle are similar in appearance to ordinary unspecialized myocardial fibres: in places these small limb fibres swell out into large Purkinje fibres (Pl. 6, fig. 22), and it would seem reasonable to conclude that Purkinje fibres are simply bundle fibres which have proceeded a step further in structural specialization. Nothing whatever has been observed in the present study to suggest that Purkinje fibres are embryonic fibres at an arrested stage of development as has been stated by Retzer (1920). At no time during their differentiation do myocardial fibres not belonging to the conducting system even remotely resemble Purkinje fibres. It is interesting to note that Erlanger (1909) found that the A.V. bundle possessed no powers of regeneration after severance, a finding which lends support to the conception of these fibres as highly specialized elements. Cady (1921), Tufts (1922) and Nonidez (1943) all agree with the present observations.

Still-born hearts

Only now do the fibres of the S.A. node possess the full characters by which they are usually identified, i.e. they are, with silver impregnation particularly, definitely cross-striated although not so strongly as the neighbouring atrial fibres; they are spindle shaped and of smaller breadth than atrial fibres and are arranged circumferentially around the nodal artery (Pl. 6, fig. 24). Groups of ganglion cells are numerous on the surface of the node and its nerve supply at birth is rich. The A.V. bundle is still relatively very large and the A.V. node has increased in vascularity, but no other features call for comment.

Foetal electrocardiography

Bell (1938) in this country and Ward & Kennedy (1942) in America succeeded in obtaining electrocardiograph tracings of the foetal heart using leads from the maternal abdominal wall. With great kindness Drs Ward & Kennedy sent the

writer a large series of their original recordings covering the last 24 weeks of term, from which it was hoped correlation might be made between the anatomical development of the conducting system of the heart and its degree of physiological activity. This, however, is not yet possible owing to technical difficulties such as the smallness of the deflexion caused by the foetal heart recorded in this way. Tracings which can be analysed, however, have been taken from foetuses removed by hysterotomy by Heard, Burkley & Schaefer (1936) and by Marcel & Exchaquet (1938). It is difficult to regard records so obtained as physiological, particularly as the heart rates are definitely less than those of foetuses *in utero*, but until improved technique allows of earlier and better intra-uterine recordings these must suffice. The authors mentioned above used direct chest leads and obtained success from very young specimens indeed. Heard *et al.* (who unfortunately did not reproduce their earliest tracings) reported their youngest as being of 9½ weeks' gestation, but the ages of Marcel & Exchaquet's specimens appear to have been assessed by a method other than that commonly used. However, from the data given it seems that an adult-type electrocardiograph tracing was obtained from an embryo of between 6 and 7 weeks' gestation and of about 16 mm. C.R. length. This is a most remarkable thing, for although the bundle and both nodes are then recognizable their differentiation is far from complete and they are quite without a nerve supply.

The conduction time throughout intra-uterine life is approximately the same as in the adult: allowing for the difference in heart size it follows that the excitation wave must at first be very much slower than in the adult, but that with increase in heart size its speed will gradually approach the adult value. Consequently Purkinje fibres begin to appear in the limbs of the bundle in order that its passage may be facilitated. The extent to which the specialized conducting tissue is a neuromuscular mechanism is hard to say, but it is probable that, although functioning early on independent of nervous control, for the proper fulfilment of its purpose an adequate nerve supply is required.

An adult-type tracing from an embryo of 16 mm. is interesting for another reason; at that stage of development, as has been noted, the A.V. bundle is not the only connexion between the atria and ventricles, and it might be expected that a tracing from such a heart would show a double ventricular wave or similar abnormality. That it does not do so is due to the very small size of the heart in which the time difference in the arrival of separate impulses to the ventricle is infinitesimal and not recordable.

DISCUSSION

It seems clear from the evidence available that structural differentiation of the specialized conducting tissue of the human heart commences about the 8 mm. stage, the A.V. node and bundle being formed in advance of the S.A. node, and that the time of appearance of nerve elements in relation to this tissue, although variously reported on, may be said to be approximately 20 mm. Now

how does this fit in with our present knowledge of the early functional development of the vertebrate heart?

Goss (1942), in rat embryos, observed the initiation of contraction occurring in embryos of three somites in which the heart consisted of two lateral rudiments. The first activity was the feeble twitching of a few cells of the left heart just to the ventricular side of the A.V. junction. The contractions had a regular rhythm of 34–42/min. and were followed in 2 hr. time by similar activity of the same part of the right heart, the 'beats' of which were independent of and slower than those of the left. By five somites the ventricle was single and contracting as a unit. Towards the end of the six-somite stage a few cells on the atrial side began to contract 0·1–0·2 sec. before the ventricle and the atrium became the pacemaker with a consequent increase in rate. At eight somites the circulation began, the co-ordination of the whole heart being made possible by the establishment of the A.V. interval which appeared along with atrial contraction and inhibited the spontaneous ventricular rhythm.

These results agree with Copenhaver's (1939) in *Amblystoma* and with those of Patten & Kramer (1933) in the chick. The latter authors especially emphasize that the change of pacemaker occurs before any neuroblasts approach the heart and before even the 'suggestion' of a specialized conducting system.

The work of Davis (1927) on the development of the human heart up to the twenty-somite stage has shown that the heart tube is formed by a progressive fusion of paired primordia as in other forms, so that in all probability development of contractility is as just described. Since in the amphibian, avian and mammalian hearts, at a time when no specialized tissue has yet developed, contraction proceeds from the venous to the arterial end with, as in the adult, an A.V. interval to ensure the co-ordination of the heart, why does the specialized tissue develop at all in birds and mammals? The reason appears to be this: Whereas the slow rate of contraction (30–40/min.) of the very young heart will persist throughout life in the Amphibia, in birds and mammals very rapid rates may develop (for example, when first detected clinically the human foetal heart rate is about 160/min.), necessitating a mechanism capable of immediately propagating the impulse throughout the heart. The cardiac conducting tissue provides this mechanism, and *pari passu* with its appearance the atria and ventricles become separated by fibrous tissue until the A.V. bundle is left as their sole connexion, thus ensuring a single rapid impulse being carried to the ventricles which therefore contract together. The speed of conduction is important, and Lewis (1925) has stated that in Purkinje tissue the excitation wave travels ten times as fast as in ordinary cardiac muscle. On the other hand, in the slowly beating amphibian heart there is no specialized tissue (Davies 1942); instead there is present at the junctional zones a peculiar arrangement of the ordinary muscle fibres (Skramlik, 1921; Davies & Francis, 1941) sufficient to cause the delay in transmission which occurs there and which corresponds to that caused by the A.V. node in higher forms.

All heart muscle possesses rhythmic activity independent of any nervous

connexions whatever, and as this is of greatest frequency at the venous end of the heart transmission of the beat normally starts from there. In order that the needs of the body as a whole may be served the automatic activity of the heart must be subject to the central nervous system. For this reason the S.A. node from a very early stage is in the closest relation with the nervous system, and by discharging its duties under nervous control ensures for mammals and birds a degree of circulatory efficiency not enjoyed by lower animals.

SUMMARY

1. The atrio-ventricular bundle can be recognized in a human embryo of 8 mm. C.R. length.

2. It arises from the A.V. node by a process of rapid growth which carries it from a position behind the dorsal endocardial cushion to the summit of the inter-ventricular septum where it divides.

3. The A.V. node represents a part of the original A.V. canal which undergoes early structural specialization.

4. Purkinje fibres of typical appearance can be recognized at the 105 mm. stage. They are myocardial fibres which have become further specialized. There is no evidence that they are fibres which have remained in an embryonic condition.

5. The sinu-atrial node can be identified at 10 mm., but only at birth does it show the histological features which characterize it in the adult.

6. The facts of foetal electrocardiography are discussed in the light of new information regarding the development of the conducting tissue.

7. The close relation borne by nerve elements to the developing S.A. and A.V. nodes is described.

8. The significance of the development of specialized conducting tissue in the mammalian heart is discussed.

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The drawing from which Pl. 1 was prepared was made by Miss M. White of the Histology Department, University College, Cardiff.

REFERENCES

Ashley, L. M. (1945). A determination of the diameters of ventricular myocardial fibres in man and other mammals. *Amer. J. Anat.* **77**, 325–357.

Bell, G. H. (1938). The human foetal electrocardiogram. *J. Obstet. Gynaec.* **45**, 802–809.

Blair, D. M. & Davies, F. (1935). Observations on the conducting system of the heart. *J. Anat., Lond.*, **69**, 303–323.

Cady, L. D. (1921). A microscopical study of the sinoventricular bundle of the rabbit's heart; with reference to the data relative to its functional interpretation, especially in terms of a source of replacement of degenerated myocardium. *Anat. Rec.* **21**, 375–385.

Calcagno, I. R. (1941*a*). Desarrollo del haz de His en embriones vacunos. *Rev. Soc. argent. Biol.* **17**, 213–220. (Read in abstract.)

Calcagno, I. R. (1941*b*). Desarrollo del nódulo de Tawara en embriones vacunos. *Rev. Soc. argent. Biol.* **17**, 221–228. (Read in abstract.)

Copenhaver, W. M. (1939). Initiation of beat and intrinsic contraction rates in the different parts of the Amblystoma heart. *J. exp. Zool.* **80**, 193–224.

Davies, F. (1942). The conducting system of the vertebrate heart. *Brit. Heart J.* **4**, 66–76.

Davies, F. & Francis, E. T. B. (1941). Conducting system of heart of *S. salamandra*. *Philos. Trans.* B, **231**, 99–130.

Davis, C. L. (1927). Development of the human heart from its first appearance to the stage found in embryos of twenty paired somites. *Contr. Embryol. Carneg. Instn*, **19**, 245–284.

Erlanger, J. (1909). Can functional union be re-established between the mammalian auricles and ventricles after destruction of a segment of the auriculo-ventricular bundle? *Amer. J. Physiol.* **24**, 375–383.

Fahr, A. (1907). Ueber die musculäre Verbindung zwischen Vorhof und Ventrikel (das Hissche Bündel) im normalen Herzen und beim Adams-Stokesschen Symptomkomplex. *Virchows Arch.* **188**, 562–575.

Frazer, J. E. (1931). *A Manual of Embryology*, p. 307. London: Baillière, Tindall and Cox.

Goss, C. M. (1942). The physiology of the embryonic mammalian heart before circulation. *Amer. J. Physiol.* **137**, 146–152.

Heard, J. D., Burkley, G. G. & Schaefer, C. R. (1936). Electrocardiograms derived from eleven fetuses through the medium of direct leads. *Amer. Heart J.* **11**, 41–48.

His, W. Jr. (1893). Die Thätikeit des embryonalen Herzens. *Arb. med. Klin. Lpz.* Cited by Mall, F. P. (1912) in *Amer. J. Anat.* **13**, 278.

Jones, D. S. (1942). The origin of the vagi and the parasympathetic ganglion cells of the viscera of the chick. *Anat. Rec.* **82**, 185–193.

Jones, Tudor J. (1932). The connexion between the cardiac nodes. *Lancet*, **2**, 389–390.

Keith, A. (1909). Malformations of the heart. *Lancet*, **2**, 519–523.

Keith, A. & Flack, M. (1906). The auriculo-ventricular bundle of the human heart. *Lancet*, **2**, 359–364.

Kent, A. F. S. (1893). Researches on the structure and function of the mammalian heart. *J. Physiol.* **14**, 233–254.

Lewis, T. (1925). *The Mechanism and Graphic Registration of the Heart Beat*, 3rd ed. London: Shaw and Sons.

Mall, F. P. (1912). On the development of the human heart. *Amer. J. Anat.* **13**, 249–298.

Marcel, M. P. & Exchaquet, J. P. (1938). L'électrocardiogramme du foetus humain. *Arch. Mal. Cœur*, **31**, 504–512.

Matsuda, K. (1936). Anatomical study of conduction system of heart in human newborn and fetus. *Jap. J. Obstet. Gynec.* **19**, 57–75.

Monckeberg, J. G. (1908). *Untersuchungen über das Atrio-ventriklär-bündel im menschlichen Herzen.* Jena: Fischer. Cited by Walmsley, T. (1929) in Quain's *Anatomy*, 11th ed. London: Longmans, Green and Co.

Nonidez, J. F. (1943). Structure and innervation of conductive system of heart of dog and rhesus monkey, as seen with silver impregnation technique. *Amer. Heart J.* **26**, 577–597.

Ohnell, R. F. (1946). *Pre-Excitation: A Cardiac Abnormality.* London: Kimpton.

PATTEN, B. M. & KRAMER, T. C. (1933). The initiation of contraction in the embryonic chick heart. *Amer. J. Anat.* **53**, 349–375.

RETZER, R. (1908). Some results of recent investigations on the mammalian heart. *Anat. Rec.* **2**, 149–155.

RETZER, R. (1920). The sino-ventricular bundle; a functional interpretation of morphological findings. *Contr. Embryol. Carneg. Instn*, **9**, 143–156.

RONDOLINI, G. (1937). Trasformazioni nella struttura del sistema di conduzione del cuore nell' uomo durante il periodo fetale e postnatale. *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **106**, 782–806.

SANABRIA, T. (1936). Recherches sur la différenciation du tissu nodal et connecteur du cœur des mammifères. *Arch. Biol., Paris*, **47**, 1–70.

SEGRE, R. (1926). Recherches sur la portion sino-auriculaire du système de conduction du cœur humain. *Arch. Mal. Cœur*, **19**, 295–302.

SHANER, R. F. (1929). The development of the atrio-ventricular node, bundle of His and sino-atrial node in the calf; with a description of a third embryonic node-like structure. *Anat. Rec.* **44**, 85–94.

SKRAMLIK, E. VON (1921). Ueber die anatomische Beschaffenheit der Ueberleitungsgebilde des Kaltblüterherzens. *Z. ges. exp. Med.* **14**, 246–281.

STIÉNON, L. (1926). Recherches sur l'origine du nœud sinusal dans le cœur des mammifères. *Arch. Biol., Paris*, **36**, 523–539.

TANDLER, J. (1912). *Manual of Human Embryology* (Keibel and Mall), **2**, 569. Philadelphia and London: Lippincott.

TAWARA, S. (1906). *Das Reizleitungssystem des Säugetierherzens. Eine anatomisch-histologische Studie über das Atrioventricular-Bündel und die Purkinjeschen Fäden.* Jena: G. Fischer.

TUFTS, J. M. (1922). Some observations upon structure of the Purkinje fibres. *Anat. Rec.* **22**, 363–372.

WALLS, E. W. (1945). Dissection of the atrio-ventricular node and bundle in the human heart. *J. Anat., Lond.*, **79**, 45–47.

WARD, J. W. & KENNEDY, J. A. (1942). The recording of the fetal electrocardiogram. *Amer. Heart J.* **23**, 64–70.

WOLFF, L., PARKINSON, J. & WHITE, P. D. (1930). Bundle-branch block with short P-R interval in healthy young people prone to paroxysmal tachycardia. *Amer. Heart J.* **5**, 685–704.

EXPLANATION OF PLATES

Abbreviations used in the figures

Ao.	Aortic valve cusp.	*P.*	Phrenic nerve.
A.V.B.	Atrio-ventricular bundle.	*P.F.*	Purkinje fibre.
A.V.N.	Atrio-ventricular node.	*R.*	Right venous valve.
C.S.	Coronary sinus.	*R.D.C.*	Right duct of Cuvier.
D.	Dorsal endocardial cushion.	*S.A.N.*	Sinu-atrial node.
G.	Nerve ganglia.	*S.Pr.*	Septum primum.
I.V.C.	Inferior vena cava.	*S.V.*	Sinus venosus.
L.	Left venous valve.	*S.V.C.*	Superior vena cava.
L.L.	Left limb of A.V. bundle.	*T.*	Tricuspid valve cusp.
M.	Mitral valve cusp.		

PLATE 1

Transverse section of 16·5 mm. human embryo to show the A.V. bundle on the summit of the inter-ventricular septum at its junction with the dorsal endocardial cushion. ×60.

PLATE 2

Fig. 1. Transverse section embryo 161, 8 mm., section 19.2.2. The A.V. bundle, stained very darkly, is situated on the summit of the inter-ventricular septum which at this point has succeeded in joining the dorsal endocardial cushion. A prolongation of the bundle down the left side of the septum can also be seen. ×18.

Fig. 2. Part of the preceding section at a higher magnification. The appearance of the bundle is that of an actively growing structure. ×280.

Fig. 3. Transverse section embryo H. 33, 10 mm., section 19.1.2. The bundle can be seen arising from the A.V. node and passing into the base of the dorsal endocardial cushion. In this section as in the next a slender strand of muscle still connects the left atrium and ventricle. ×30.

Fig. 4. Transverse section embryo H. 33, 10 mm., section 18.2.6. To show the continuation of the bundle down the left side of the inter-ventricular septum. ×30.

PLATE 3

Fig. 5. Part of section 18.2.6. of embryo H. 33. To show vacuole formation in some of the cells of the primitive left limb of the A.V. bundle—the forerunners of Purkinje fibres proper. ×1000.

Fig. 6. Transverse section embryo H. 33, 10 mm., section 17.1.4. A cell cluster in the base of the right sinus valve represents the future S.A. node. ×30.

Fig. 7. Sagittal section embryo 44, 18·8 mm., section 23.2.5. To show the S.A. node within the substance of the crista terminalis. ×15.

Fig. 8. Transverse section embryo 114, 25 mm., section 38.2.2. The A.V. node is here shown close to where it will give origin to the bundle. ×40.

PLATE 4

Fig. 9. Transverse section embryo H. 23, 13 mm., section 25.3.5. To show the kidney-shaped mass forming the S.A. node within the base of the venous valves. ×150.

Fig. 10. Transverse section embryo H. 23, 13 mm., section 26.2.3. To show the left limb of the A.V. bundle passing into trabeculae (false tendons) of the left ventricle. The relation of the bundle to the septal cusp of the tricuspid valve is well seen. ×30.

Fig. 11. Transverse section embryo H. 23, 13 mm., section 26.1.8. To show the first identifiable strands of the right limb of the bundle. ×30.

Fig. 12. Transverse section embryo H. 23, 13 mm., section 26.2.7. To show the space which has now developed around the bundle. ×150.

PLATE 5

Fig. 13. Heart of 40 mm. embryo, section 24.1.5. To show the position of the S.A. node between the sinus and atrium. ×20.

Fig. 14. The previous section at a higher magnification. Nerve elements can be seen entering the anterior part of the node. ×75.

Fig. 15. Heart of 40 mm. embryo, section 27.2.4. To show the A.V. bundle cut in coronal section on the summit of the inter-ventricular septum between the mitral and tricuspid valves. ×20.

Fig. 16. Heart of 40 mm. embryo, section 26.2.5. To show the bundle dividing. ×20.

Fig. 17. Part of the previous section to show the finer structure of the right limb of the bundle. ×650.

Fig. 18. Heart of 40 mm. embryo, section 29.2.4. To show a leash of unmyelinated nerve fibres which runs into the A.V. node. ×650.

PLATE 6

Fig. 19. Heart of 105 mm. embryo, section 42.3. The inter-ventricular septum is here shown in vertical section. ×50.

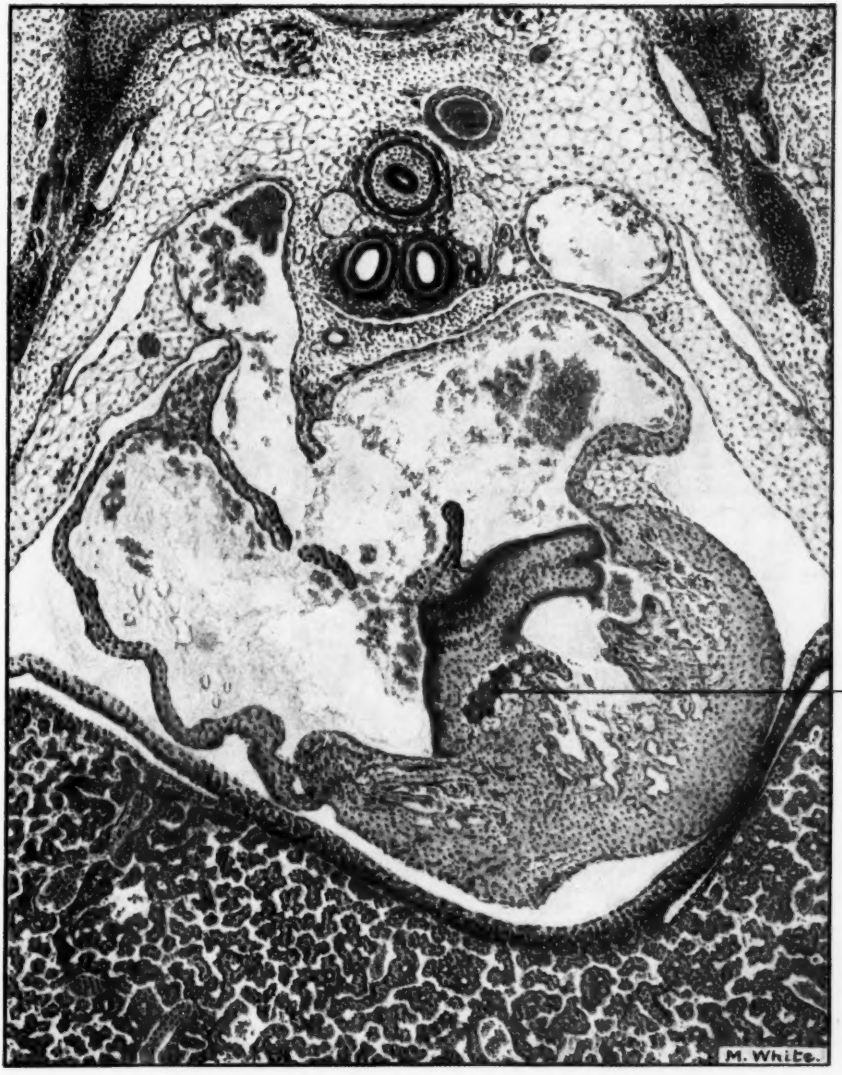
Fig. 20. Heart of 123 mm. embryo, section 7.2.2. Nerve ganglion of the posterior atrial wall. ×350.

Fig. 21. Heart of 150 mm. embryo, section 14.4. Vertical section of the inter-ventricular septum to show the left limb arising from the main bundle with no evidence of the right limb. This is the commonest type of division, the right limb seeming really the continuation of the bundle. ×100.

Fig. 22. Heart of 150 mm. embryo, section 14.4. Part of the left limb of the A.V. bundle is here shown cut longitudinally. It contains a few Purkinje fibres and in the top right corner of the photomicrograph an apparently unspecialized myocardial fibre can be seen swelling out into a large Purkinje fibre containing two nuclei. Endocardial surface above and to the left. ×300.

Fig. 23. Part of the same left limb shown in the previous figure. Many large, typical Purkinje fibres are shown in which cross-striation is marked. Endocardial surface to the right. ×450.

Fig. 24. Transverse section of sinu-atrial region of a still-born heart, section 9.1.2. Endocardial surface above and to the right. ×60.


A.V.B.
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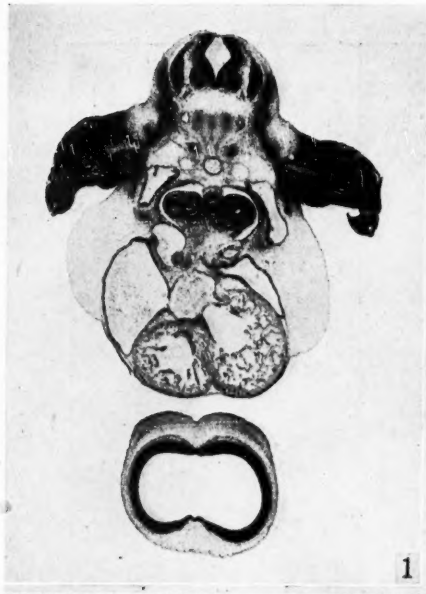

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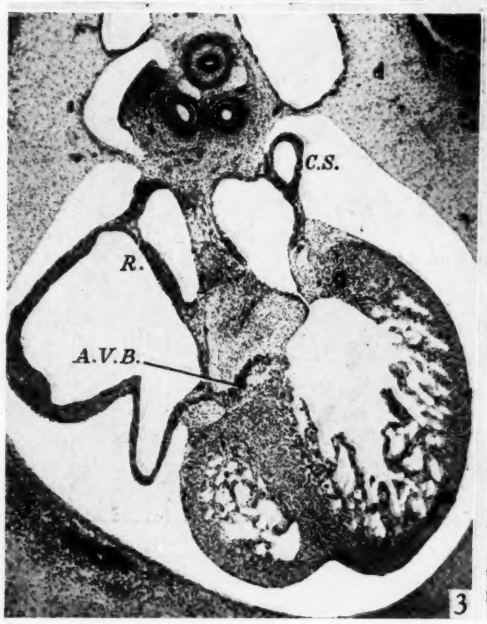

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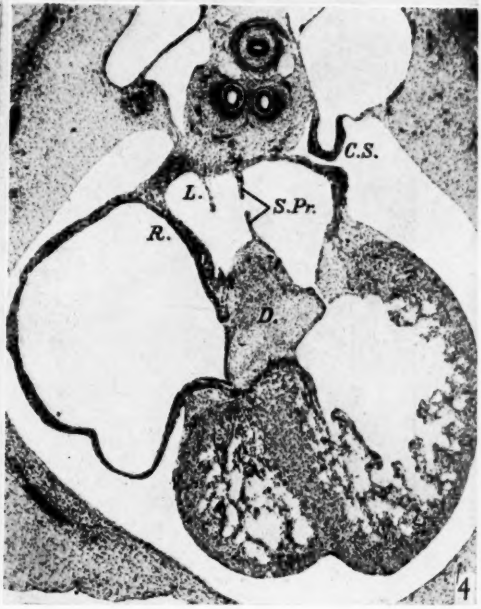

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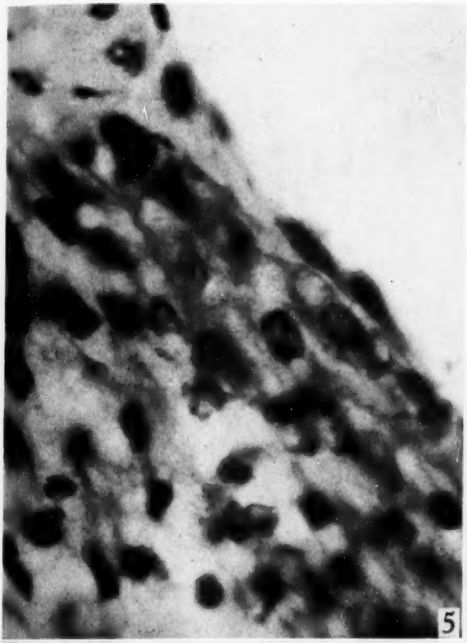
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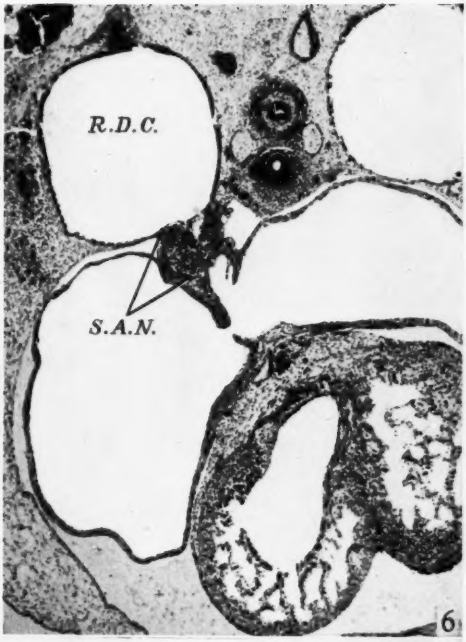

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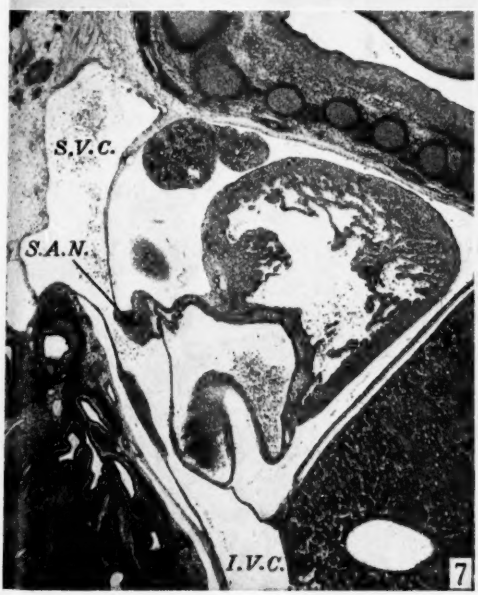

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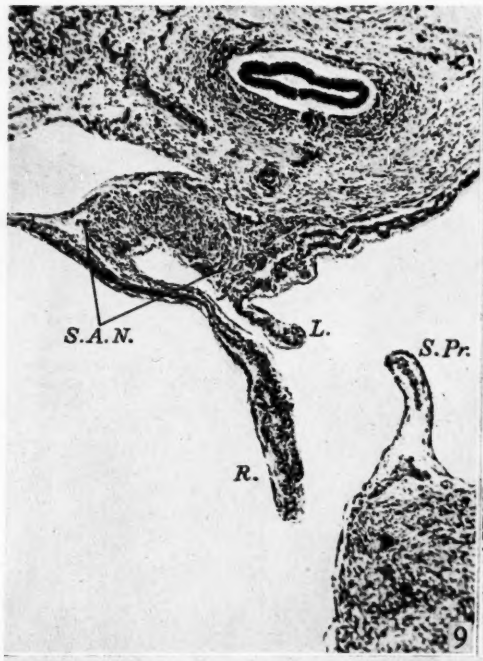
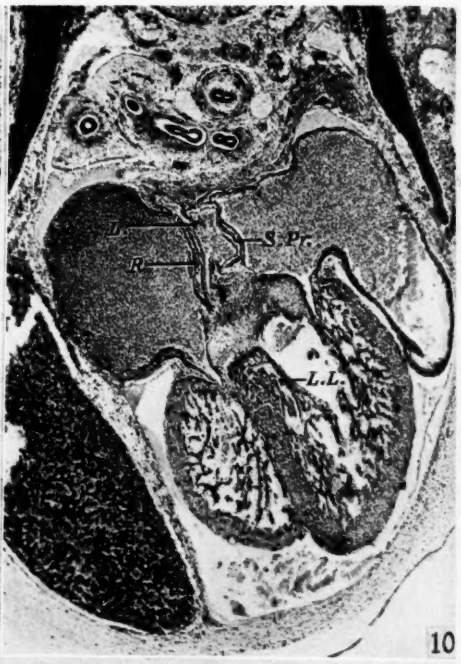
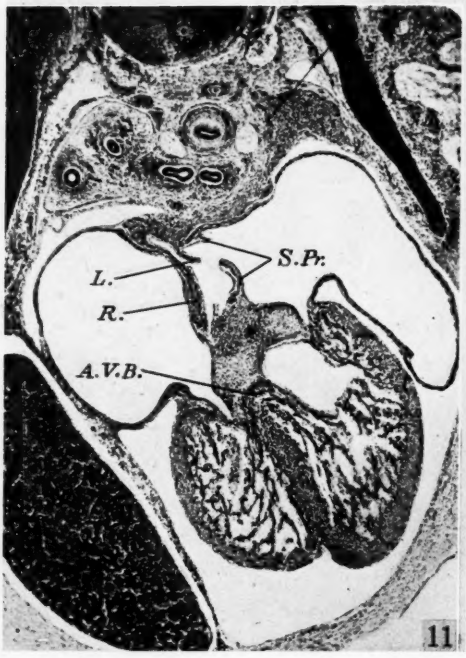
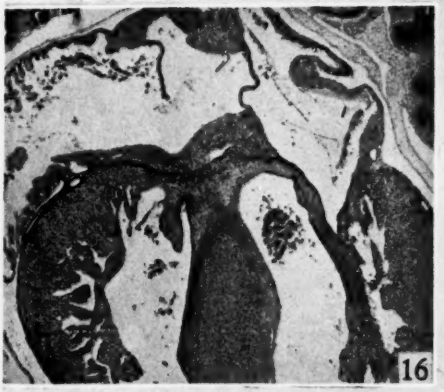
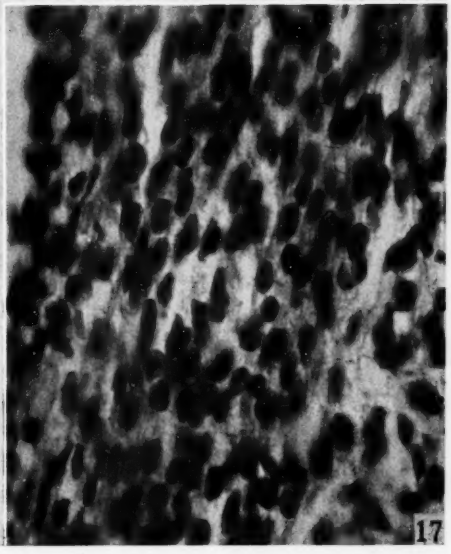


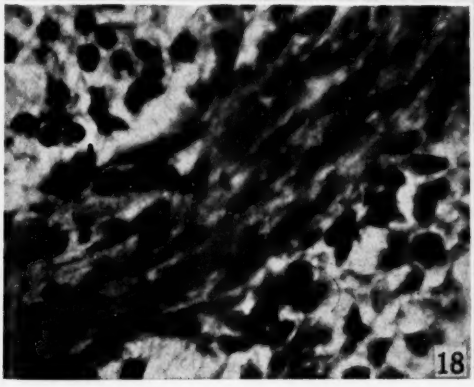




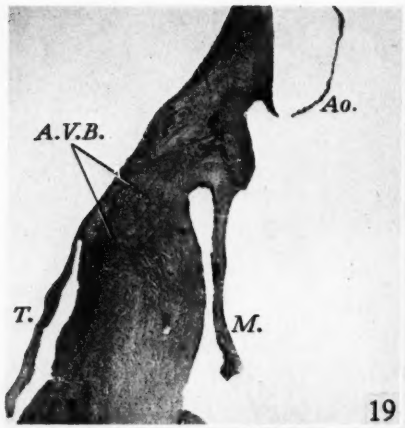
WALLS—Specialized conducting tissue of the human heart

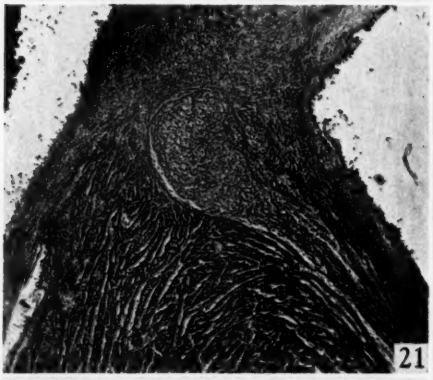


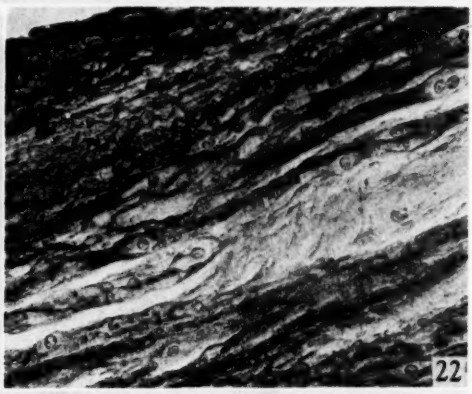


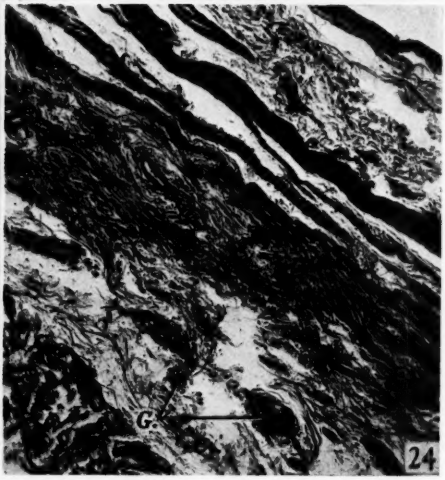


WALLS—Specialized conducting tissue of the human heart









WALLS—SPECIALIZED CONDUCTING TISSUE OF THE HUMAN HEART

THE RADIOGRAPHIC DEMONSTRATION OF THE MODE OF ACTION OF THE HEART OF THE FROG

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INTRODUCTION

It has been widely held, and indeed it is stated as a fact in most text-books of zoology, that while the heart of the frog has an undivided ventricle it acts nevertheless in such a way that the so-called arterial and venous blood mix very little. Thus the blood returning from the lungs is said to be sent mainly to the carotid vessels while some goes to the systemic circulation, and the blood coming from the general venous circulation is sent mainly to the pulmo-cutaneous vessels though some mixes in the systemic arches with that from the lungs. It has thus been maintained that the so-called oxygenated blood from the lungs is sent mainly to the head region, and that the venous blood is excluded from the carotid circulation.

The origin and growth of this classical hypothesis has been traced by Vandervael (1933), who produced experimental evidence that it is untenable and that in the ventricle considerable, and possibly complete, mixing of arterial and venous blood takes place. He pointed out that this mixing is to be considered as being consistent with the mode of life of the frog, for it is well known that whether the frog is in water or on land considerable cutaneous respiration takes place. Vandervael's method was to inject Indian ink in saline into the pulmonary vein and to observe its distribution in the three aortic arches which he illuminated from below.

Foxon (1947) has recently confirmed Vandervael's results by a method which, it is claimed, is free from certain objections which can be levelled against the same author's technique. Foxon also made injections through the cutaneous vein into the right atrium and found that the results were not detectably different from those obtained when the injections were made through the pulmonary veins and left atrium. These observations strengthen the mixing hypothesis very considerably. Foxon has also pointed out that the mixing hypothesis is in keeping with our present knowledge of the evolutionary history of the vertebrate heart.

However, the injection of Indian ink suffers from the disadvantage that while the results may be convincing to the operator they are not readily demonstrable; neither can they be preserved in any form of photographic or other permanent record. It was, therefore, decided to attack the problem by the introduction of a radio-opaque medium into the blood stream and to examine its subsequent course by means of X-rays.

This paper gives an account of the technique evolved and of the results obtained. It may be stated at the outset that in our opinion these results are quite inconsistent with the classical hypothesis and point very strongly to considerable mixture taking place in the heart.

While both authors have collaborated at all stages of the research one of us (E.W.W.) has paid particular attention to the radiographic techniques involved, and the other (G.E.H.F.) has been mainly responsible for the operative work and injections; the interpretation of the radiographs has been in all cases agreed by both authors.

MATERIAL AND METHODS

Foxon (1947) found no difference to be apparent in the mode of action of the hearts of frogs and toads; they have both, therefore, been used in the present investigation. The frogs were *Rana temporaria* and *R. esculenta* and the toads *Bufo bufo*. Pithed frogs and toads were injected with contrast media and, either photographs were taken, or observations were made by direct screening.

The method used in making the injections is that described in detail by Foxon for injecting Indian ink, and may be summarized thus: 'Solila' hypodermic needles for use with cartridge-type syringe (Amalgamated Dental Co. Ltd.) 26 gauge by 1½ in. in length were used. The double-ended needle, previously filled with contrast medium, was inserted into the appropriate vessel under a dissecting magnifier, and was kept in position by being pressed into a block of plasticine. The other end of the needle was then pushed into the rubber tube of the injection syringe thus making the injection unit complete. The syringe used was an 'Agla' micrometer injection apparatus (Burroughs Wellcome and Co.) to which a convenient length of rubber tubing had been attached, the tubing being closed with a glass bung. One complete turn of the micrometer screw of the syringe was stated by the makers to deliver 0·00996 c.c. Suitable precautions were taken to see that the fluid in the injection apparatus was not under any pressure which would cause premature injection of the contrast medium.

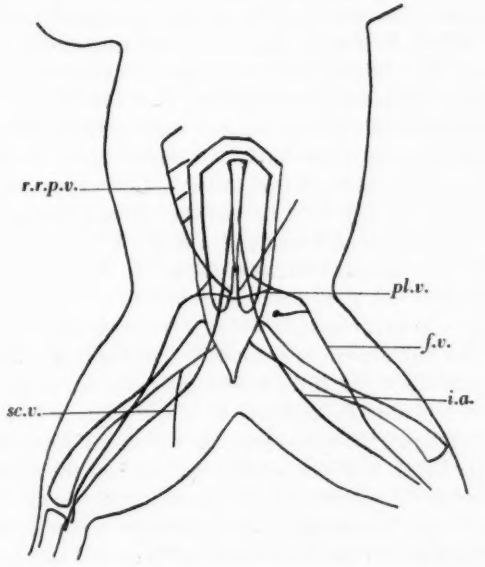
When the preparation was complete it was set up on the couch of the X-ray apparatus and either photographs were taken or direct observations made.

The X-ray apparatus is a Watson Sunic Roentgen Power Unit. For the photographs the tube distance was 28 in. and the exposures varied from 0·15 to 0·2 sec. using a current of 30 mA. and 60 kV.

Photographs were usually taken in series of three. This was done by means of a wooden slide, the two ends of which were covered by lead sheeting. Between the two sheets of lead was a gap over which the animal was supported on a cork dissecting board. The gap was of such a size that the shadow of the whole animal was projected on to the cassette lying immediately below. It was possible to push cassettes from under one piece of lead to the correct position under the animal for exposure in sequence, and in this way a series of exposures could be made quite conveniently at about 3 sec. intervals. However, as a

timing device the beating heart of the frog, exposed in the dissection, was used, exposures being made, for example, on the fifth, tenth, and fifteenth heart beats after the injection was begun. This method obviated any complications which might have arisen from the fact that the rate of heart beat in the frog varies with the temperature of the surroundings.

The following contrast media were tried; 'Uropac', a proprietary brand of Uroselectan B; 'Perabrodil', a proprietary medium containing bromine salts, and 'Thorotrast'. Of these thorotrast only proved suitable for the present study. This substance is a colloidal suspension of thorium dioxide miscible with



Text-fig. 1. Tracing of radiograph of *Rana esculenta* showing presence of thorotrast in the renal portal vessels (approximately natural size). For other details see text. The abbreviations used are the same as those for the plates.

blood. It has no apparent effect, chemical or mechanical, on the frog's heart and the colloidal particles are small enough to pass through the capillaries. This last point is made clear in Text-fig. 1 which is a tracing of a radiograph which showed thorotrast in the renal-portal vessels of a specimen of *Rana esculenta*. The animal had been used for screening and had been injected with a comparatively large amount of thorotrast over some ten minutes, the photograph being taken about a quarter of an hour after screening was finished.

The beating of the heart was also used to time the injection; normally half a turn of the micrometer screw of the injection apparatus was given at each heart beat during the injection (ten such half-turns were usually the maximum

at one time), each such half turn giving 0·005 c.c. approximately. As to how this volume compares with the total volume of the ventricle reference may be made to the description of the frog's heart given by Clark, Eggleton, Eggleton, Gaddie & Stewart (1938). From these workers' figures (although the heart weight varies considerably in relation to the size of the animal), taking males and females of different sizes, the average weight for the heart of *R. temporaria* is 0·0685 g. Of this the ventricle accounts for some 70 % or 0·048 g.

Again, according to these authors, a ventricle of 0·11 g. will, when distended, hold about 0·2–0·3 c.c. of fluid. Therefore a ventricle of half the size might be expected to hold 0·1–0·15 c.c. Now the ventricle of the frog does not completely empty itself at each heart beat, and the amount of residual blood would appear from the work of Clark *et al.* to be about one-tenth of the total; thus if a ventricle held 0·1 c.c. of fluid, 0·09 c.c. would be taken in during each beat. If, therefore, into such a heart were introduced 0·005 c.c. this would amount only to one-eighteenth of the anticipated total cardiac intake. Because a certain amount of blood is unavoidably lost during the preliminary operation and the insertion of the needle, it seems most unlikely that the amount experimentally introduced in any way upsets the normal pressure relationships existing in the heart and neighbouring vessels. We believe that this conclusion is justified by the photographs themselves which, in the case of pulmonary vein injections, never show any sign of the contrast medium flowing across into the other pulmonary vein, as it certainly would if the pressure were too great. Also the shadow of the left atrium is extremely significant as it always presents a concavity towards the right; if there were any question of excess pressure this concavity would probably be replaced by a convexity where the interatrial septum would be bulging the wrong way. In the case of injections through the venous side of the heart, however, we must admit that the medium, no matter how carefully injected, did tend very frequently to flow in the wrong direction as well as towards the sinus venosus. We have no evidence, however, that this in any way affected the action of the heart, or that the heart tended to take in more than its normal volume at each beat.

OBSERVATIONS

The classical hypothesis of the mode of action of the heart of the frog, already referred to in the introduction, rested upon several suppositions. These have been shown to be incorrect in so far as they concern the passage of the blood up the two sides of the spiral valve of the truncus and the sequence of filling of the arterial arches. These events were supposed to be separated in time; in fact they have been found by Vandervael (1938) to be simultaneous, and this observation has been confirmed by Foxon (1947). Therefore there only remains the obvious suggestion that the anatomical arrangements of the heart and great vessels are, in themselves, sufficient to keep separate the blood returning from the lungs and to direct it mainly into the carotid circulation. Both Vandervael and Foxon

have held that this is not so, and it is the validity of their assertions which is, we believe, demonstrable by radiographic methods. A secondary point on which these methods give evidence also is the degree of mixing taking place in the ventricle, for the old view was that the blood from each atrium tended to remain distinct in the ventricle. The results will be presented under the following headings: (*a*) pulmonary vein injections; (*b*) vena cava injections; (*c*) direct observations by screening.

(*a*) *Pulmonary vein injections.* If the old hypothesis were correct, then when an injection is made through the pulmonary vein most of the contrast medium should go to the head region, some to the systemic circulation and none to the pulmo-cutaneous vessels.

A series of injections was obtained using a toad (Pl. 2, figs. 5, 6). Fig. 5 shows the condition after injection has been made for five heart beats; the bases of the carotid arteries and a considerable length of the right subclavian artery are clearly visible. Five beats later (fig. 6), by which time the medium is well seen in the vessels of the head region, it is also quite clearly seen in the right lower limb as far as the knee, and it is also well seen in the lung.

Thus there is no sign of the blood which has arrived in the left atrium being selected and sent to the head. Comparable series have been obtained in the frog. It might be objected that the contrast medium seen in the pulmo-cutaneous vessels has already returned through the general circulation into the right atrium but no evidence of this has been found.

A series in the frog is shown in Pl. 1, figs. 1–3. This series is not easy to interpret; the amount of contrast medium was almost minimal and for some undetermined reason the vessels on the animal's left side do not show so clearly as those on the right. This series is included because it shows the type of variation encountered. The only vessels to be clearly filled from the outset are the systemic arch and the dorsal aorta. The carotid vessels do not give any marked shadows but the right pulmonary artery gives a somewhat indefinite shadow indicated in fig. 3. This series by itself proves nothing but it serves to illustrate a point made by Foxon (1947) concerning his Indian ink injections, viz. that if a very small quantity of ink is injected it is visible only in the systemic vessels. Whilst dealing with the variability of these results it must be pointed out that almost the only result we have not achieved is that required by the old hypothesis, namely that the carotid vessels should be filled more clearly than the others.

(*b*) *Vena cava injections.* The term 'vena cava injection' is used to include not only injections made through the anterior venae cavae themselves but also through vessels leading into them.

As has already been noted, in the injections through the venous side of the heart, trouble was experienced with back flow of the contrast medium. These retrograde injections have, however, been of considerable assistance in the interpretation of other photographs.

Injection into a tributary has been preferred to injection into the vena cava

itself as it was thought that any tendency to force fluid into the heart might be avoided by the fluid flowing peripherally in the veins.

A very clear series is shown in Pl. 2, figs. 7–9. Here the injection was made through the left musculo-cutaneous vein. According to the old view the injection should show most clearly in the pulmo-cutaneous arteries. However, the contrast medium shows most clearly in the systemic vessels and by the time (fig. 9) that the pulmonary and cutaneous arteries can be clearly identified so can the carotid vessels, which, of course, should not be visible at all (see also fig. 4).

(*c*) *Screening.* Although screening was carried out with great care it was never possible to identify individual vessels. It was possible, however, to see the heart shadow very clearly. This is of importance with regard to the degree of mixing in the ventricle. No difference in the ventricular shadow could be discerned between pulmonary and vena caval injections. In both instances it had a recognizable conical form, e.g. figs. 4 and 9. After injection was stopped the ventricle cleared the contrast medium in about four or five heart beats. This showed that the amount of residual fluid in the heart was not very great (a point already mentioned), and also that the thorotrast showed no tendency to 'stick' to the ventricular wall. Our impression is that as the ventricle expands in diastole, blood from both atria finds its way to all parts of the ventricle. The fact that thorotrast injected through the pulmonary vein does not fill one side only of the ventricle is shown in figs. 1–3, where it is clearly visible on both sides in various stages of the cardiac cycle. Owing to the fact that the sinus venosus and anterior venae cavae underlie the ventricle to a large extent, it is not so easy to draw conclusions on this point from the photographs of the vena cava injections, but figs. 8 and 9 show the medium well distributed in the whole of the ventricle.

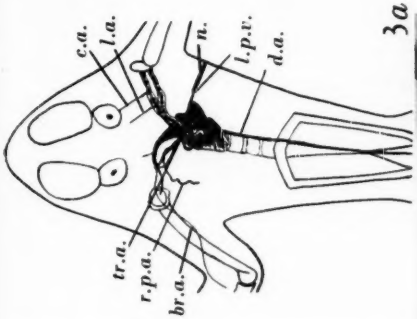
CONCLUSION

As a result of the photographic study made and the direct screening observations undertaken, the conclusion has been reached that the methods of X-ray investigation adopted give no support whatever to the old hypothesis of selective distribution of blood by the hearts of frogs and toads. In spite of some variation in the results it is concluded that they are sufficiently consistent to maintain the view that considerable mixture of blood from left and right atria takes place in the ventricle.

SUMMARY

1. A technique for the injection of radio-opaque contrast media into the circulation of pithed frogs and toads is described.

2. This method demonstrates that the classical hypothesis, which holds that the blood of these amphibians is selectively distributed so that only blood returning from the lungs is sent to the head, is untenable.



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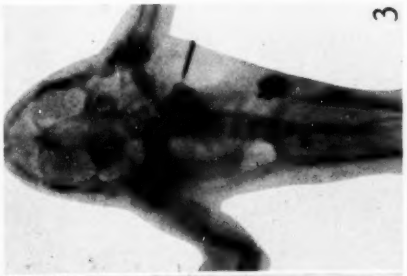

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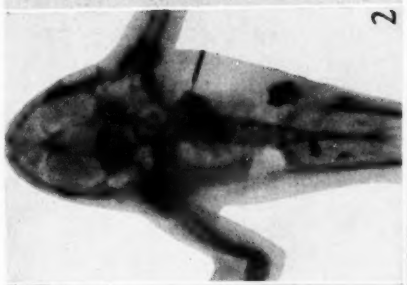

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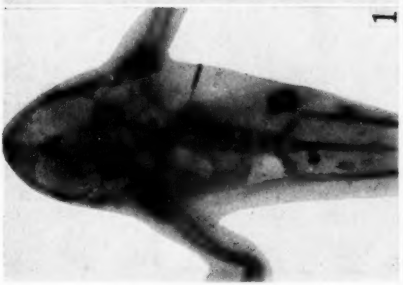

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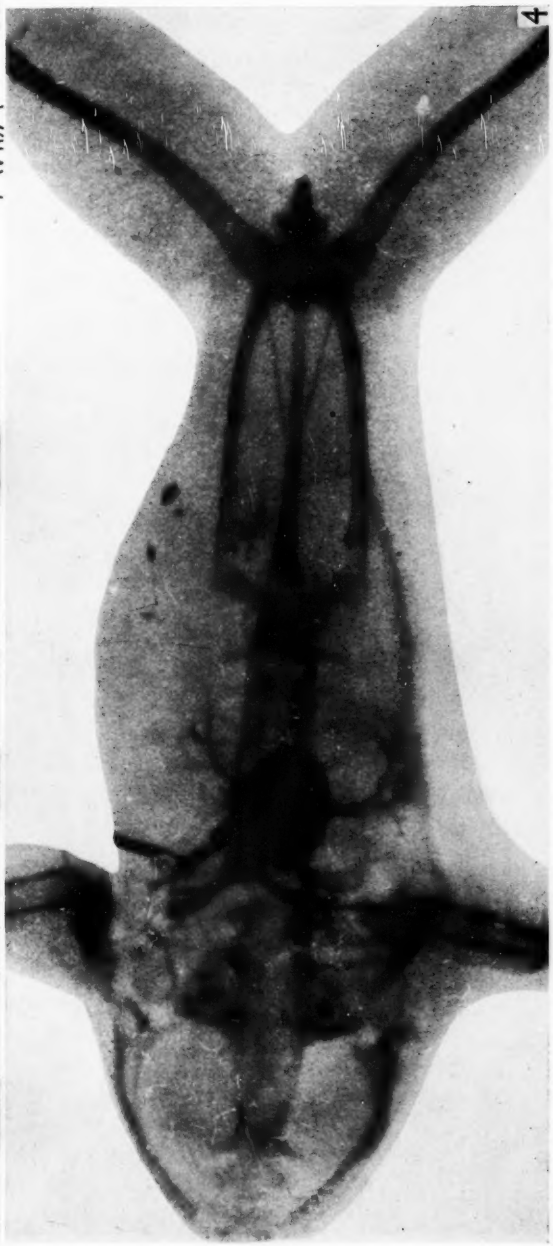

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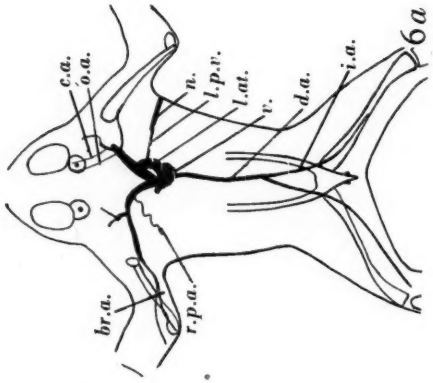
FOXON AND WALLS—THE MODE OF ACTION OF THE HEART OF THE FROG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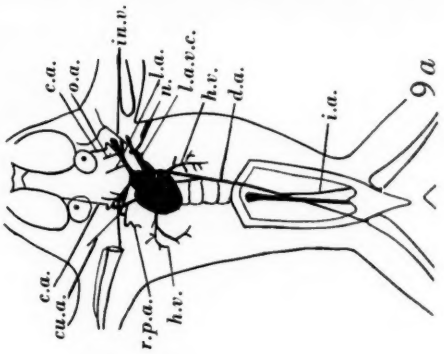
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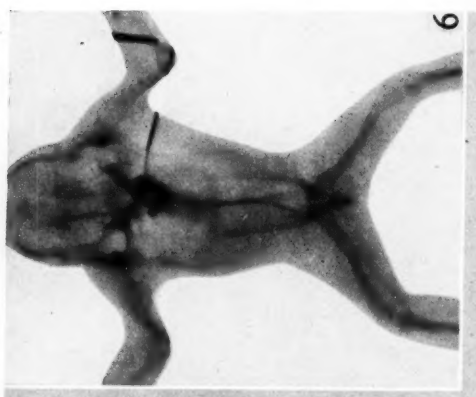
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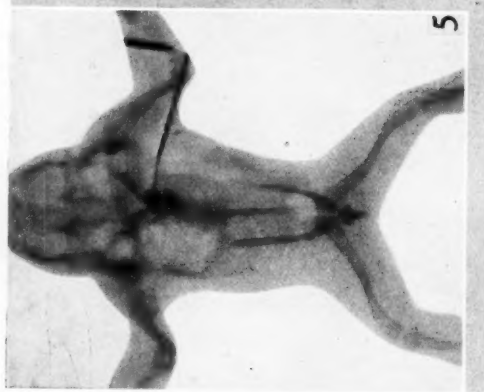
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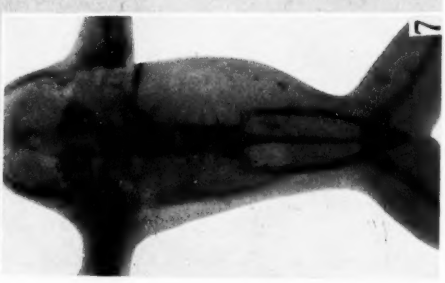
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3. Injection either through the pulmonary or anterior vena caval streams reveals considerable mixture of blood in the ventricle.

4. Mixture in the ventricle has been confirmed both by screening and photography.

We wish to thank Prof. C. M. West for allowing us to carry out this work in his department and for according us full radiographic facilities. We also gratefully acknowledge the great help given by Mr A. Welch in making the photographs.

REFERENCES

CLARK, A. J., EGGLETON, M. G., EGGLETON, P., GADDIE, R. & STEWART, C. P. (1938). *The metabolism of the frog's heart.* Edinburgh and London: Oliver and Boyd.

FOXON, G. E. H. (1947). The mode of action of the heart of the frog. *Proc. zool. Soc. Lond.* (in the Press).

VANDERVAEL, F. (1933). Recherches sur le mechanisme de la circulation du sang dans la cœur des amphibiens anoures. *Arch. Biol., Paris*, **44**, 577–606.

EXPLANATION OF PLATES

Abbreviations used in figures

br.a.	brachial artery.	*l.at.*	left atrium.
c.a.	carotid artery.	*l.p.v.*	left pulmonary vein.
cu.a.	cutaneous artery.	*n.*	injection needle.
d.a.	dorsal aorta.	*o.a.*	occipito-vertebral artery.
f.v.	femoral vein.	*pl.v.*	pelvic vein.
h.v.	hepatic vein.	*r.p.a.*	right pulmonary artery.
i.a.	iliac artery.	*r.r.p.v.*	right renal portal vein.
in.v.	innominate vein.	*sc.v.*	sciatic vein.
l.a.	lingual artery.	*tr.a.*	truncus arteriosus.
l.a.v.c.	left anterior vena cava.	*v.*	ventricle.

(All figures, except Pl. 1, fig. 4, are direct prints from radiographs reproduced natural size.)

PLATE 1

Figs. 1, 2, 3 and 3*a*. Series of radiographs, with key, showing an injection of a specimen of *Rana temporaria* through left pulmonary vein. Thorotrast was injected on heart-beats 1–10 and photographs taken on heart-beats 5, 10 and 15. The principal vessels are indicated in the 'key', fig. 3*a*.

Fig. 4. *Rana temporaria*. An enlargement ($\times 2\frac{1}{4}$) of Pl. 2, fig. 9.

PLATE 2

Figs. 5, 6 and 6*a*. *Bufo bufo*. Radiographs showing injection through the left pulmonary vein, with key. Thorotrast injected on heart-beats 1–10; photographs taken on heart-beats 5 and 10. In fig. 5 the trabecular structure of the ventricular wall is well marked. The closed atrio-ventricular valve is also clearly seen.

Figs. 7, 8, 9 and 9*a*. Series of radiographs of specimen of *Rana temporaria* showing injections through left musculo-cutaneous vein. Thorotrast injected on heart-beats 1–15. Photographs taken on heart-beats 5, 10 and 15. In this series retrograde injection occurred (see text) into the hepatic and innominate veins.

RESPIRATORY DISPLACEMENT OF LARYNX, HYOID BONE AND TONGUE

BY A. G. MITCHINSON AND J. M. YOFFEY

From the Department of Anatomy, the University, Bristol

The present brief study of laryngeal movement during respiration arose out of a consideration of the movements of the heart and diaphragm in respiration. During deep inspiration the central tendon of the diaphragm may often be seen to descend 1–1½ in., and sometimes as much as 2 in. In man the pericardium is firmly attached to the central tendon, and therefore must descend at the same time, and the heart with it. But the heart does not descend as a whole, for while the lower part of the heart descends within the pericardium, the upper part, with the great vessels, remains relatively fixed. The aortic arch, for instance, undergoes in the majority of cases scarcely any vertical displacement on deep breathing. The reason for this fixation is not altogether clear. Strong sterno-pericardiac ligaments could be responsible in part, but not infrequently these ligaments are poorly developed. The hooking of the great vessels round the bronchi, the aorta round the left, and the pulmonary arteries round both right and left, might be effective, but only if the lung root were relatively fixed.

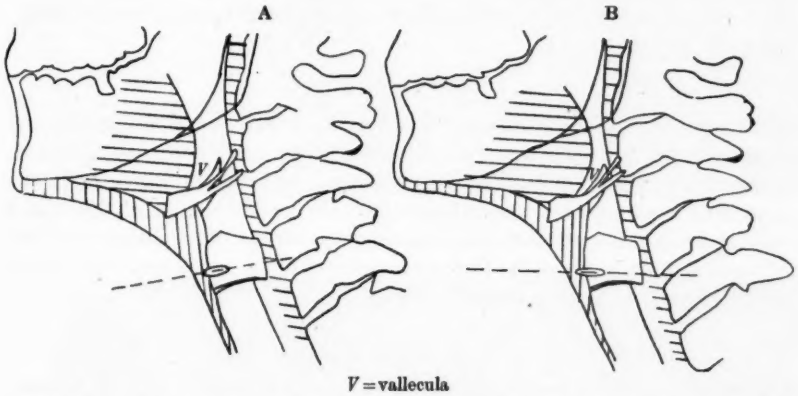
But, is the lung root fixed? According to Macklin (1925) the extent of the vertical movement of the lung roots is about ½ in., which must mean either a corresponding descent or actual stretching of the trachea by that amount. But descent of the trachea would involve descent of the larynx also, and the purpose of the present study was to find out whether such displacement of the larynx does normally occur. In aortic aneurysm the diseased vessel can certainly displace the trachea and larynx, giving rise to the well-known 'tracheal tug'.

Twenty-three subjects from 15 to 45 years of age, and of both sexes (fifteen male, eight female) were X-rayed in extremes of inspiration and expiration. The subjects were standing, the head being clamped, jaws closed, and the back resting against a wooden board, so that there should be no movement of head and vertebral column. In spite of these precautions it was found difficult in a few cases completely to avoid movement, but such movement was never very marked. The distance from tube to subject was 5 ft.

RESULTS

In five cases there was elevation of the larynx during inspiration, in four cases descent, the maximum movement being ¼ in., and in the remaining fourteen cases there was no appreciable vertical displacement. The hyoid bone underwent a vertical displacement corresponding to that of the larynx (Pl. 1).

Presumably the non-descent of the larynx during inspiration is due to contraction of the supra-hyoid muscles (mylo-hyoid, stylo-hyoid, posterior belly of digastric) as well as stylo-pharyngeus and thyro-hyoid. Digital examination seems sometimes to indicate that this contraction occurs on deep inspiration. If this is so the trachea during inspiration is subjected to traction at both ends, and after an initial elongation of about $\frac{1}{2}$ in. should become more tense during inspiration. The supra-hyoid muscles might thus be regarded as important accessory muscles of inspiration, and through them and the stylo-pharyngeus and the muscles and ligaments connecting hyoid bone, laryngeal cartilages and trachea to one another, the trachea is slung from the base of the skull. Strong stylo-hyoid ligaments would reinforce the sling in its upper part, while the middle and inferior constrictors could also assist. If this interpretation be correct, then ultimately it is the contraction of the supra-hyoid and



V = vallecula

Text-fig. 1. Female aet. 18. Tracing from X-ray photograph. A = full inspiration, B = full expiration.

Note. (*a*) Forward movement of the tongue and hyoid bone in deep inspiration. (*b*) Practically no vertical displacement of the larynx. (*c*) Forward rotation of the larynx in deep inspiration, as indicated by the dotted line which passes through the laryngeal sinus. (*d*) Change in outline of epiglottis and widening of vallecula.

other muscles mentioned above that is responsible to a variable extent for preventing the root of the lung and the trachea from descending after its elasticity has been expended. This contraction might be in the nature of a stretch reflex.

The trachea as seen in the dissecting room in fixed subjects, has usually lost its elasticity. But a fresh trachea obtained in the post-mortem room is elastic, and can be easily elongated by about $\frac{1}{2}$ in., but not more even when considerable force is applied. Its extensibility appears to possess a fairly sharp end-point, after which the trachea undergoes no further increase in length.

Though in the present study there was no constant vertical displacement of the larynx, an unexpected movement of the tongue and hyoid bone was encountered during respiration. In the majority of cases (18 out of 23, 78 %) there was a forward movement of the tongue and hyoid bone in full inspiration, averaging $\frac{3}{8}$ in. The purpose of this hyolingual displacement is doubtless to facilitate the ingress of air into the respiratory passages (Text-fig. 1 and Pl. 1) particularly during deep inspiration. The genio-hyoid and genio-glossus would thus become in many persons accessory muscles of inspiration.

It was also found (*a*) that the distance between larynx and hyoid increased by an average of only $\frac{1}{10}$ in. in full inspiration; (*b*) that in ten of the subjects there was a forward tilting of the larynx in full inspiration, the average movement being 8° (Text-fig. 1); and (*c*) that the epiglottis underwent a characteristic movement. The part attached by the hyo-epiglottic ligament to the hyoid bone moves forwards with that bone, but the margins above that are fixed by the ary-epiglottic folds. Hence the vallecula becomes wider (Text-fig. 1 and Pl. 1).

SUMMARY

In twenty-three persons the larynx was X-rayed in extremes of inspiration and expiration. In five cases there was elevation of the larynx during inspiration, in four cases descent, the maximum movement being about $\frac{1}{4}$ in., and in the remaining fourteen there was no appreciable vertical displacement. The hyoid bone underwent a vertical displacement like the larynx. In eighteen cases out of twenty-three, appreciable forward movement of the hyoid bone and tongue and epiglottis occurred in deep inspiration.

REFERENCE

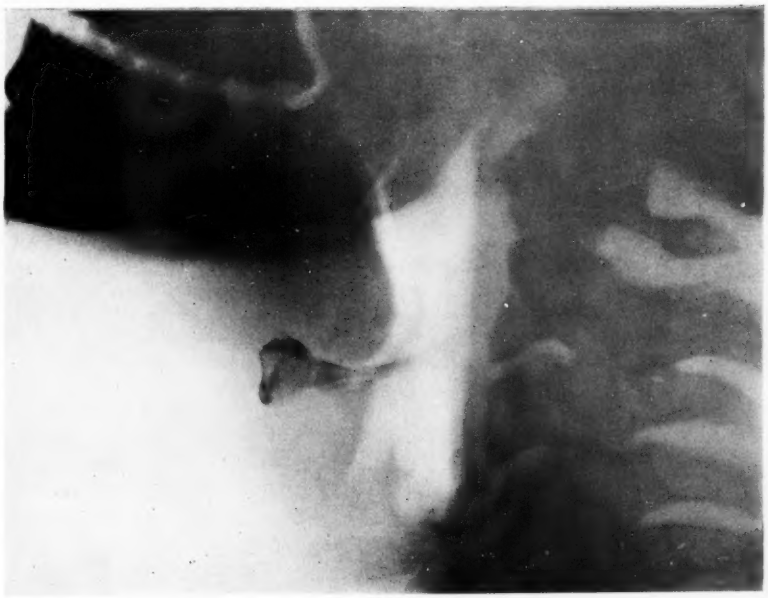
Macklin, C. C. (1925). X-ray studies on bronchial movements. *Amer. J. Anat.* **35**, 303–329.

EXPLANATION OF PLATE

Female aet. 28. A = full inspiration, B = full expiration.

Note. (*a*) Forward movement of tongue and hyoid bone in deep inspiration. (*b*) Hyoid bone and larynx $\frac{1}{4}$ in. lower in deep inspiration probably due to downward pull of lung root.

A



B

MITCHINSON AND YOFFEY—RESPIRATORY DISPLACEMENT OF LARYNX, HYOID BONE AND TONGUE

REVIEWS

The Anatomy of the Bronchial Tree (with special reference to the surgery of lung abscess). By R. C. Brock, M.S. (Lond.), F.R.C.S. (Eng.). (Pp. vi+96; 142 illustrations; med. 8vo; 42*s*. net.) London: Oxford University Press, Geoffrey Cumberlege.

A clear and interesting account of the lobes of the lungs and their relation to the skeleton and to the branches of the bronchial tree. This book is well produced and beautifully illustrated with radiographs, photographs, line and coloured drawings.

Anatomical Eponyms. By Jessie Dobson, B.A., M.Sc. Foreword by F. Wood Jones. (Pp. ix+240; frontispiece; fcap. 4to; 30*s*. net.) London: Baillière, Tindall & Cox.

A well-printed and attractively produced biographical dictionary giving interesting personal and familial information about nearly 750 persons whose names have been associated with anatomical structures. There is a frontispiece of Vesalius.

The Principles of Anatomy, an Introduction to Human Biology. By A. A. Abbie, M.D., B.S., D.Sc., Ph.D. 2nd ed., revised and enlarged. (Pp. x+273; 76 figs.; demy 8vo; 12*s*. 6*d*.) Sydney and London: Angus & Robertson Ltd., obtainable at all booksellers.

A book to 'illustrate human structure by consideration of the problems which had to be solved by the human as a living animal maintaining itself in a largely hostile environment', and intended for those with little or no biological training, and based on the belief that 'biological principles could be illustrated as well from the human as from any other animal'. This edition contains sections on genetics and growth gradients and there is a short history of Anatomy and a glossary. There are a few references.

Practical Anatomy. By W. E. Le Gros Clark, M.A., D.Sc., F.R.S., F.R.C.S. (Pp. xvi+470; 251 illustrations; demy 8vo; 25*s*. net.) London: Edward Arnold & Co.

This, as the author explains in his Preface, is a rewritten, revised, re-arranged and pruned version of the 1932 edition of *Practical Anatomy, by Six Teachers* (719 pp.), published by Edward Arnold, with the object of eliminating factual data not required by 95% of medical students. The 1932 edition has been supplemented by instructions for preliminary and supplementary studies which are suggested as a suitable guide to the extent of the knowledge required of the student in the oral tests which, in most departments of Anatomy, are given during the course of each dissection. Revision has affected chiefly the section on the brain.

Gray's 'Anatomy', Descriptive and Applied, edited by T. B. Johnston, C.B.E., M.D., and J. Whillis, M.D., M.S. 29th ed. (Pp. xix+1597; 1359 illustrations, 642 coloured and 47 X-ray plates; med. 8vo; 70*s*. net.) London: Longmans, Green & Co.

A carefully revised and partly rewritten edition of the well-known text-book. Rewriting has affected chiefly the section on Embryology, and Neurology, and a number of new X-ray plates have been added.

The Circulation in the Foetus. By KENNETH J. FRANKLIN, D.M., F.R.C.P., ALFRED E. BARCLAY, O.B.E., D.M., F.R.C.P. and MARJORIE M. L. PRITCHARD, M.A. (Pp. iv + 28; 13 illustrations; demy 8vo; paper, 2*s.* 6*d.* net.) Oxford: Blackwell Scientific Publications.

An abbreviated edition of the same authors' book *The Foetal Circulation*, published by Blackwell in 1944. It is intended as a synopsis for medical and veterinary students and for nurses in training, also for senior folk who are interested but have not the time for perusal of the original book.

A Synopsis of Surgical Anatomy. By ALEXANDER LEE MCGREGOR, M.Ch. (Edin.), F.R.C.S. (Eng.) With a foreword by Sir HAROLD J. STILES. 6th ed. (Pp. xiii + 714; 699 illustrations; cr. 8vo; 25*s.* net.) Bristol: John Wright & Sons Ltd.

A revised edition of this well-known handbook. Contains a great variety of concise information and adorned with simple but effective diagrams.